INFLUENCES OF FERTILITY LEVELS, HOST AND PATHOGEN VARIABILITY, AND INOCULATION TECHNIQUE ON PITCH CANKER DISEASE IN SLASH PINE

BY

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1989

ACKNOWLEDGEMENTS

I am grateful to Dr. George M. Blakeslee who was willing to dedicate his time, patience, and expertise to my graduate program. His counsel and belief in my abilities provided the leadership responsible for me to achieve this goal. Most rewarding, however, was his receptive and responsive demeanor in the development of this research.

I am appreciative to the members of my supervisory committee for their guidance and understanding: Drs. Nicholas B. Comerford, Susan V. Kossuth, Thomas Miller, Donald L. Rockwood, and Robert A. Schmidt.

I am especially appreciative to Dr. Robert A. Schmidt for his counsel, insight, and invaluable advice. I am grateful to Dr. Ken Portier for providing statistical expertise, and to Dr. Roger Webb for sharing his provisions in times of need.

I thank Joel Smith for lending his assistance, experience, wisdom, and for maintaining levity in all aspects of the research. I am grateful to Renee Holley for her assistance and support, to Julie Klapproth for her unfaltering assistance during the most trying of times, to Winnie Lante whose assistance was invaluable and good hearted nature made working with her a pleasure, to Sarah Mesa for her laboratory assistance and whose spirit endeared her to all, to George Barker for his assistance and humor, and to B. J. Rabe for her clerical support. I especially thank both Pat Layton for her friendship and generosity in helping with the project and Holly Krysko for her understanding and friendship. The field

aspect of this research would have not been possible without the generous offer of Earl M. Underhill, Forest Manager, Miami Corporation, for the research site and technical insight. In addition, I thank Mary Mcleod and CRIFF for the foliar analysis performed, and the Soil Testing Laboratory for the soil analysis.

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Abstract of Dissertation Presented to the Graduate School of
the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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May, 1989

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Genetic and environmental factors regulating the expression of pitch canker disease in slash pine were examined. Host response to pitch canker fungal isolates, inoculum concentration, wounding technique, and season of inoculation was evaluated among four mature slash pine seed orchard clones. Influences of nitrogen and phosphorus fertilization rates, and nitrogen form, on symptom expression of slash pine progenies inoculated with different pitch canker fungus isolate sources were evaluated in a separate field study.

Wounding by puncturing the outer bark of branches with a needle provided consistent exposure of aqueous conidial suspensions of Fusarium subglutinans to host tissue for reliable evaluation of pitch canker resistance. Inoculum concentrations of 1 X 10^4 conidia/ml and greater were required for consistent host response. The amount of branch dieback increased with increasing inoculum concentrations

but was only significant for the most susceptible clone. Amounts of branch dieback did not differ between summer and fall inoculation trials.

Differences in virulence among inoculum sources of Fusarium subglutinans, evaluated as the amount of branch dieback response to inoculation, were significant, although considered minor and unrelated to geographic differences in stand disease expression. Host clones of intermediate response significantly interacted with inoculum source indicating the importance of inoculum selection in evaluating host resistance.

The frequency of branch dieback following inoculation of young, field-grown slash pine progeny significantly increased with increasing application rates of ammonium sulfate (168.12 and 448.32 kg/ha N [150 and 400 lbs/ac]), but only when applied in combination with concentrated superphosphate (112.08 kg/ha P [100 lbs/ac]). Branch dieback was positively related to foliar concentrations of both N and P, although the influences of foliar P concentrations were independent and very strong compared to N. Both the amount of branch dieback and foliar concentrations of N and P were similar between treatments receiving 448.32 kg/ha $N\text{-}NO_3$ (400 lbs/ac) and 168.12 kg/ha $N\text{-}NH_4$ (150 lbs/ac) indicating influences of N form on disease expression are more related to N assimilation.

Pitch canker resistance in slash pine appeared under strong genetic control, attenuated by environmental conditions. The physiological basis for pitch canker resistance appears related to the rate of host containment of pathogen invasion and not to the predisposition to wounding.

Deployment of pitch canker resistant slash pine genotypes in combination with thoughtful use of forest fertilization practices could provide effective pitch canker management strategies even in areas of high disease hazard.

CHAPTER I

INTRODUCTION

Pitch canker disease of slash pine (Pinus elliottii Engelm. var. elliottii), caused by pine pathogenic strains of Fusarium subglutinans (Wollenw. & Reinking) Nelson, Tossoun & Marasas comb. nov., is characterized by conspicuous resinous cankers occurring on branches or on the main stem. Typically, the canker expands, girdling the stem and causing dieback of distal portions of the branch. Numerous infections within a tree can result in severe crown dieback and tree mortality; lesser levels of branch dieback can result in growth loss.

Disease outbreaks in slash pine stands have been localized, sporadic in occurrence and location, and characterized by rapid disease intensification and subsequent reduction (Bethune and Hepting 1963, Laird and Chellman 1972, Schmidt and Underhill 1974). Major epiphytotics occur less often but are also characterized by rapid changes in disease conditions (Dwinell and Phelps 1977, Phelps and Chellman 1976, Schmidt 1976).

In slash pine, disease expression appears to be mediated by several response-regulating environmental factors and by the genetic relationships of host-pathogen interactions.

Increases in site fertility levels (fertilization of seed orchards, proximity to farming operations, etc.) are often associated with increases in pitch canker infections in slash pine stands (E. L. Barnard, personal communication, Cleason

and Smith 1978, Phelps and Chellman 1976). Field and greenhouse studies indicate that increases in pitch canker disease expression are generally associated with N fertilization (Fisher et al. 1981, Fraedrich and Witcher 1982, Lowerts et al. 1985, Wilkinson et al. 1977). Evaluations of different rates and forms of available N sources on pitch canker have not been reported although Huber and Watson (1974) conclude N form is generally more influential in plant disease than is the rate of N application.

Genetic variation in pitch canker susceptibility has been reported for both clones and half-sib progenies of slash pine (Blakeslee and Rockwood 1978, Dwinell and Barrows-Broaddus 1979, Lowerts et al. 1985, McRae et al. 1985, Rockwood et al. 1988). Increases in the appearance of new pitch canker infections in slash pine during the fall (G. M. Blakeslee, personal communication) and reported increases in branch dieback in loblolly pine from fall-winter inoculations (Kuhlman et al. 1982) suggest corresponding seasonal changes in host susceptibility.

Significant differences in virulence of the pitch canker fungus are not reported (Barrows-Broaddus and Dwinell 1979, 1983, 1984, Blakeslee et al. 1980b, Dwinell and Barrows-Broaddus 1983, Kuhlman et al. 1982). Differences were reported, however, when Virginia pine seedlings were inoculated with isolates obtained from different loblolly pine tissues (Barrows-Broaddus and Dwinell 1985).

Other factors influencing the phenotypic expression of pitch canker may include the concentration of inoculum to which a tree is exposed and the inoculation technique. Numerous inoculation techniques and inoculum concentrations have been used in previous studies (Barrows-Broaddus and Dwinell 1983, Blakeslee et al. 1980b, Dwinell 1978, Fraedrich and Witcher 1982). Inoculation techniques have included wounding by slitting the bark of the shoot, chisel wounds to the bole, needle punctures of the shoot epidermis, and the removal of

needle fascicles. Inoculum introduced into these wounds consisted of aerial fungus mycelium, growth media supporting fungal colonies, and aqueous conidial suspensions of varying concentrations.

Intensive management of slash pine in the southern U.S. affords the opportunity to utilize genotypes selected for resistance to disease and to alter site fertility through operational fertilization. By 1986, 0.3 million ha of commercial forest lands planted to slash pine were fertilized (Allen 1987). Evaluation of the influences of forest fertilization on disease expression of slash pine genotypes inoculated with selected pitch canker fungal isolates could be useful in developing potential pitch canker disease management strategies. Understanding the contribution of the response-regulating factors (host genotype, fungal isolate, inoculum concentration, wounding technique, and season of inoculation) in disease expression could provide insight into the disease biology and enhance the development of reliable and consistent inoculation methodologies for use in future pitch canker research and in selection and screening for pitch canker resistance.

The objectives of this study were to 1) evaluate the response of slash pine genotypes to inoculum sources and concentrations of the pitch canker fungus; 2) determine the influence of artificial inoculation procedures (i.e., season of inoculation and technique of wounding) on disease expression; 3) characterize host response to inoculation related to genetic mechanisms of resistance; 4) determine the influences of nitrogen and phosphorus application rates, and forms of nitrogen, on host-pathogen interactions of the slash pine-pitch canker fungus pathosystem; 5) evaluate the relationship of host nutrition and height growth response with disease expression.

CHAPTER II

REVIEW OF THE LITERATURE

History

In the spring of 1945, Hepting and Roth (1946) observed lethal girdling cankers at the base of branches and leaders of Virginia pines (Pinus virginiana Mill.) growing near Asheville, North Carolina. This canker disease was distinct from other known cankers of southern pines because of the copious pitch production and accumulation on and below the cankers, and the complete resin soaking of the subtending xylem. These symptoms led Hepting and Roth to propose the name 'pitch canker'. Shortleaf (P. echinata Mill.) and pitch pine (P. rigida Mill.) were similarly affected in the same stand but to a lesser extent. The authors also noted the occurrence of a similar canker on slash pine (P. elliottii Engelm. var. elliottii) in Georgia and Florida.

Isolation from the cankered areas produced a species of Fusarium Link ex Fr. The specific identity of the pathogen was not determined, but it possessed characteristics of the section Liseola. Several years later the causal pathogen was identified as Fusarium lateritium (Nees) em Snyd. & Hans. f. sp. pini Hepting (Snyder et al. 1949). In 1978, the identity was revised to F. moniliforme Sheld. var. subglutinans Wollenw. & Reinking (Kuhlman et al. 1978). The taxonomy of the fungus underwent further revision and is now recognized as F. subglutinans (Wollenw. & Reinking) Nelson, Tossoun & Marasas comb. nov. (Nelson et. al. 1983).

Shortly after their initial observations, Hepting and Roth (1953) reported the presence of the pitch canker disease in 38 counties in eight southern states and in Haiti. In 1959, it was suggested that pitch canker was an exotic pathogen to the United States introduced from Haiti (Berry and Hepting 1959). Dwinell et al. (1981) later concluded that the pitch canker fungus was probably endemic to the southeastern United States because Fusarium subglutinans has a broad host range and a worldwide distribution.

The ability of the pathogen to induce copious resin production led to attempts in the late 1940s to utilize the fungus in naval stores production (Hepting 1947, 1954, True and Snow 1949). Applications of sulfuric acid to chipped faces gave consistently higher gum yields, however, and the use of the pitch canker fungus in naval stores production was abandoned (Clapper 1954).

Between 1946 and 1973, reported disease outbreaks in slash, south Florida slash (P. elliottii Engelm. var. densa Little & Dorman), and Virginia pine stands were limited and erratic in time and space (Bethune and Hepting 1963, Laird and Chellman 1972, Schmidt and Underhill 1974). Such outbreaks were quite transient in nature, characterized by rapid increases in disease incidence followed by rapid decline. Mortality was low, and after several years, infections were difficult to detect.

In 1974, pitch canker became epidemic in many slash pine plantations and seed orchards in Florida (Dwinell and Phelps 1977, Phelps and Chellman 1976, Schmidt 1976). Incidence of infected trees exceeded 90% in some individual slash pine plantations with 25% pitch canker-induced tree mortality (Blakeslee and Oak 1979). Similar levels of pitch canker infection were also reported on loblolly pine (P. taeda L.) in seed orchards in North Carolina and Mississippi (Dwinell et al. 1977).

Although the major epiphytotic in east-central Florida has subsided, the most recent estimate of pitch canker disease incidence indicates over 0.25 million ha of slash pine, mostly on lands in Florida and southeast Georgia, have at least 5% of the trees infected (Oak et al. 1982). Surveys on the Apalachicola National Forest, between 1978 and 1984, have documented an increase in both the percentage of slash pine stands sustaining pitch canker infection and the amount of infected trees within these stands (Oak 1984).

Since 1980, pitch canker has emerged as a problem in plantations and seed orchards of other species. Kuhlman and Cade (1985) reported a severe outbreak of shoot dieback on loblolly and pond pine (P. serotina Michx.) plantations in eastern North Carolina. Thirty-nine percent of loblolly pines and 45% of pond pines in plantations were infected. Kelly (1982) observed more than 50% pitch canker-induced mortality in a shortleaf pine seed orchard in Alabama. In Tennessee, Oak et al. (1983) reported that 98% of the ramets in a shortleaf pine seed orchard were infected by pitch canker. More than one-third of these ramets sustained over 25% crown dieback.

Several overviews on the subject of the pitch canker disease complex are published (Blakeslee et al. 1980a, Dwinell et al. 1985, Dwinell et al. 1981).

Symptomatology

Natural Infections

The pitch canker fungus can infect a variety of vegetative and reproductive host structures at all stages of development. In slash pine, strobilus mortality and deterioration of seed can result from pitch canker infection of female strobili

and mature cones (Miller and Bramlett 1979). Symptoms of infected loblolly cones include purple discoloration of green cone scales sometimes associated with surface growth of fungal mycelium on badly deteriorated cones, necrotic cone tips associated with internal pitch pockets, and occasional resinous lesions (Dwinell et al. 1985).

In forest tree nurseries, pitch canker infection can kill slash pine seedlings (Barnard and Blakeslee 1980). Diseased seedlings may appear as off-color with yellow green needles, having wilted succulent tissues and drooping of the growing tip, or with bright red-brown foliage. Lesions typically occur near the soil line with resin impregnating the adjacent xylem in advanced stages of infection.

First year slash pine outplants killed by pitch canker infections express symptoms similar to those observed on seedlings in nurseries. Pitch canker-caused field mortality is usually associated with seedlings produced in nurseries where the disease was present prior to lifting.

On larger trees, pitch canker infections typically appear as resinous cankers on the terminal, lateral branches, or main stem (Berry and Hepting 1959, Blakeslee et al. 1980a, Dwinell et al. 1985). The cankered areas usually appear sunken with the bark adhering to the necrotic regions.

On slash, south Florida slash, longleaf, loblolly, sand, Virginia, shortleaf, and eastern white pine, the xylem subtending the pitch canker infection is consistently resin impregnated (Artman 1973, Berry and Hepting 1959, Blakeslee et al. 1980b, Dwinell et al. 1977). The degree of resin soaking is quite variable in loblolly pine, ranging from slight wedges of pitch-soaked xylem tissue to almost complete impregnation (G. M. Blakeslee, personal communication, Dwinell et al. 1977). External resinosis is minimal on loblolly and on smaller branches of sand

pine in contrast to the copious flow in the other pine species, but infections on larger diameter sand pine shoots may produce large quantities of external resin.

Shoot dieback from natural infections usually occurs in the upper crowns on dominant lateral branches and terminals on loblolly, slash, and shortleaf pines in seed orchards, and on slash, loblolly and pond pine in plantations (Dwinell and Phelps 1977, Dwinell et al. 1977, Kelly and Williams 1982, Kuhlman and Cade 1985). This dieback is important in seed orchards because seed cones are borne on these shoots. Blakeslee and Oak (1979) reported more than 96% of the pitch canker infections occurred within the crowns of heavily infected slash pines. Of those infections, 76% were located in the upper two-thirds of the crown. Crown dieback, and ultimately tree mortality, can occur when a large proportion of shoots within the crown become infected and die.

Expanding lesions may be detected on shoots where one or more needle fascicles are dead and fresh resin is present (Dwinell et al. 1977). The cambium and young phloem turn a bright red-brown in the infected region and the pathogen can be readily isolated from such areas. Once the shoot is girdled by the fungus all needles distal to the infection desiccate and die. Dead needles often persist at the infection site when embedded in the resin flow.

Dieback of small diameter shoots commonly develops in late fall, during winter, or in the early spring (Blakeslee et al. 1980a, Dwinell et al. 1977). The fully expanded needles wilt, turning bright reddish-brown. Dead needles may persist for more than a year, turning dull greyish-brown with age. This difference in needle color makes it possible to distinguish current shoot mortality from previous years.

Cankers on larger diameter branches and terminals may not girdle and kill the shoot during the growing season when infection was initiated (Blakeslee et al.

1980a, Dwinell et al. 1977, Kuhlman et al. 1982). When shoot girdling occurs after initiation of growth in the spring, the new candles droop, turn brown, and die. The resulting 'shepherds crook' can be a helpful diagnostic feature. In loblolly pine seed orchard trees, Kuhlman et al. (1982) observed that severity of stem infection and the proximity of the infection to the buds determined how long the buds expanded before wilting. During the growing season, these infections caused bud mortality, wilting and dying of the current growth.

The pitch canker fungus can also infect portions of the main stem and large branches. These infections are uncommon in infected slash pine plantations (Blakeslee and Oak 1979) but are more common on pines in seed orchards and urban plantings (Blakeslee et al. 1980a). Bole cankers on slash pine in seed orchards in Florida and southeast Georgia are commonly associated with wounds made by mechanical tree shakers used to dislodge mature cones (Dwinell and Phelps 1977). Pitch canker infections on slash and longleaf (P. palustris Mill.) pines greater than five inches in diameter are seldom girdling, although smaller stems are often killed (Berry and Hepting 1959). Bole cankers are commonly found on Virginia pines of any size and often continue to expand until the main stem is girdled (Berry and Hepting 1959). A similar pattern of disease development has been observed in sand pine seed orchards. In both cases, the symptomatology is similar to lesions on smaller sized host tissue; however, large portions of tree crowns are destroyed when these large cankers eventually girdle the stem or branch.

Most new pitch canker infections are first detectable in late summer and fall on slash pine in Florida, although new infections can occur any time in the year (G. M. Blakeslee, unpublished). With loblolly pine in North Carolina seed orchards, Kuhlman et al. (1982) observed most pitch canker infections occurred in

the spring on the youngest stem tissue. Inoculations involving the same ramets did not demonstrate that susceptibility was related to host shoot phenology but did indicate that infections could occur throughout the year. Infections from artificial inoculations in fall and winter caused the greatest shoot mortality.

Artificial Inoculation

Although not commonly observed with naturally occurring pitch canker infections, responses of artificially inoculated pine shoots include formation of callus tissue and stem discoloration preceding shoot dieback. Symptoms reported by Dwinell (1978) on inoculated slash, Virginia, and shortleaf pine seedlings were resin production and reddish-purple discoloration of the stems. Comparing these same symptoms among seedlings of four susceptible pine species, Barrows-Broaddus and Dwinell (1983) noted inoculated Virginia and slash pine seedlings had more continuous resin flow and greater purple stem discoloration than loblolly and pond pine seedlings. Stem discoloration on older inoculated branches, similar to the typical seedling response, was also observed in 11-yr-old Virginia pine (Barrows-Broaddus and Dwinell 1984). These symptoms are not common on older slash pines but have been occasionally noted on seedlings in nurseries.

A margin of hypertrophic callus tissue surrounding infected tissue on living branches is another symptom observed in response to inoculation, and occasionally, in association with naturally occurring infections in slash pine (G. M. Blakeslee, personal communication). Because the hypertrophic callus response is not normally associated with shoot mortality, naturally occurring callus symptoms are infrequently noted.

In 1978, Blakeslee (personal communication) observed containment of pathogen invasion in shoots of seedlings and mature slash pines following inoculation with the pitch canker fungus. Colonization of host tissue was restricted within the margin of hypertrophic callus. Limited resin flow and impregnation of the xylem within the callus-defined cankers were commonly observed. In many cases, the host was capable of 'healing over' the restricted areas of infected tissues although the fungus remained viable for several months in the necrotic tissues.

Fraedrich and Witcher (1982) also reported callus tissue surrounding cankers following wound inoculations on the bole of 7- to 12-yr-old field-grown slash and loblolly pine. Callus symptoms were observed predominantly on trees growing on poorer sites.

Symptoms similar to the callus-like tissue produced in older trees were reported by Barrows-Broaddus and Dwinell (1983, 1984) in young shoots of inoculated slash, loblolly, Virginia, and pond pine seedlings. Inactive lesions, similar to wounded controls, formed after 56 days on inoculated 2-mo-old shoots of the 1-yr-old pine seedlings. These lesions affected less than 50% of the shoot circumference and produced little resin, in contrast to active lesions that affected more than 50% of the shoot circumference and had continued resin exudation. Compared to loblolly and pond pine, inoculated slash and Virginia pines expressed the greatest shoot mortality and had the lowest percentage of inactive lesions. In the inactive lesions, pathogen ingress was apparently halted by the formation of callus barriers in the pith, inner cortex, and parenchymatous tissues proximal to the inoculation site.

Host Range

Pitch canker disease occurs only on tree species in the genus Pinus L. This disease occurs throughout the southern United States on slash pine, south Florida slash pine, longleaf pine, shortleaf pine, and Virginia pine (Bethune and Hepting 1963, Hepting and Roth 1953). Loblolly pine, first not considered a host species (Hepting and Roth 1953), then an unusual host attacked by a specialized strain of the pitch canker fungus (Hepting 1971), is now recognized as a susceptible host species growing in seed orchards (Dwinell 1976, Dwinell et al. 1981) and plantations (Kuhlman and Cade 1985). Infections are reported on both the Ocala and Choctawhatchee varieties of sand pine (P. clausa (Chapm.) Vasey var. clausa Ward. and var. immuginata Ward., respectively) (Blakeslee et al. 1980b). Other susceptible pine hosts include Scots pine (P. sylvestris L.), pitch pine, table-mountain pine (P. pungens Lamb), pond pine, eastern white pine (P. strobus L.), and Monterey pine (P. radiata D. Don) (Artman 1973, Hepting 1961, 1971, Hepting and Roth 1953). Artificial inoculations of Japanese red pine (P. densiflora Sieb. & Zucc.), white spruce (Picea glauca (Moench) Voss), Sitka spruce (P. stichensis (Bong.) Carr.), and eastern hemlock (Tsuga canadensis (L.) Carr.) were not successful (Hepting and Roth 1953).

Host Variability

Researchers have interpreted differences in observed frequencies of naturally occurring pitch canker infections to be related to differences in susceptibility. When using artificial inoculation techniques to guard against disease escapes and to provide equal inoculum exposure, differences in pitch canker susceptibility

have been evaluated as differences in host response (e.g., amount of branch dieback, canker length, etc.).

Interspecific Host Variation

Comparing the amount of shoot mortality of inoculated 1-yr-old seedlings, Dwinell (1978) rated shortleaf and Virginia pines highly susceptible, slash pine intermediate, and loblolly pine more resistant to infection. Results from isolate virulence trials showed that Virginia pine was more susceptible than loblolly pine (90% and 36% shoot mortality, respectively) (Barrows-Broaddus and Dwinell 1979). In a more comprehensive species evaluation, Dwinell and Barrows-Broaddus (1980) concluded Virginia pine is highly susceptible to pitch canker; slash, loblolly, shortleaf, and pitch pines are moderately susceptible; and pond and eastern white pines highly resistant. In 1983, Barrows-Broaddus and Dwinell reported that half-sib families of Virginia and slash pine were more susceptible than loblolly and pond pines. The percentage of shoot mortality was unexpectedly low (10-37%) compared to their previous reports.

Variation in susceptibility among host species is also suggested by field observations. During the mid-1970s disease outbreak in east central Florida, pitch canker incidence in plantations and natural stands of south Florida slash pine (var. densa) was considerably lower than in plantations of the typical slash pine (var. elliottii) growing in the same locale (Phelps and Chellman 1976). The authors contend either genetic differences in the varieties, site effects, or their interaction as the reason for these differences.

Intraspecific Host Variation

Tree to tree variation in susceptibility to the pitch canker fungus was initially suggested by Bethune and Hepting (1963) to account for differences in infection among nearby trees in a stand during a pitch canker disease outbreak. Currently, intraspecific variation is reported for Virginia, slash, and loblolly pines.

Virginia pine. Clonal variation in pitch canker incidence was observed among Virginia pine seed orchard trees (Barrows-Broaddus and Dwinell 1984). Eight clones were ranked according to the percentage of ramets naturally infected (0-52%) by the pitch canker fungus. One-year-old, half-sib seedling families from the same eight seed orchard parent ramets were classified according to relative resistance based on the percentage of shoot mortality from two inoculation trials. Although the overall difference in shoot dieback within each trial was statistically significant, differences were not significant among most of the eight families. The rankings of the most resistant families changed between trials, but the same family was most susceptible in both evaluations. The ranking of observed infection in the seed orchard clones to the ranking of shoot dieback among the half-sib families was moderately correlated.

In the same study, 11-yr-old full-sib progeny representing four of the same clones as above, were inoculated with a different technique in two trials a year apart. Significant differences in branch dieback occurred between the two studies for the four families evaluated. Differences among the families within each trial were not strongly distinct. Family comparisons in the percentage of shoot mortality to corresponding clones in the seed orchard survey were unrelated in ranking.

Natural pitch canker infection was evaluated for three years in a Virginia pine progeny test (Barnett and Thor 1978). No significant difference in disease incidence was detected among families; however, incidence was highly significant among crosses within families. From these results the authors concluded pitch canker disease resistance in Virginia pine is inherited in a specific manner, suggesting dominance or epistatic gene action.

Loblolly pine. Clones, half-, and full-sib families of loblolly pine are reported to differ significantly in susceptibility to pitch canker. Shoot dieback was considered clonal with 90%-100% incidence among ramets for several clones in a heavily pitch canker-infected loblolly pine seed orchard (Dwinell 1976). In the apparently resistant clones, only a few dead laterals were present, while ramets in clones identified as susceptible sustained up to 50% crown dieback.

Dwinell et al. (1977) reported variation in susceptibility among loblolly clones of different geographic origins in two heavily pitch canker-infected seed orchards. In the North Carolina seed orchard previously studied (Dwinell 1976), pitch canker incidence was greater in ramets of clones from a Piedmont source than in sources from Mississippi-Alabama, and north and south Coastal Plains sources. In the other seed orchard in Mississippi, pitch canker incidence was greater in clones from south-central Arkansas and north-central Louisiana than in clones from other regions in Arkansas, Louisiana, Mississippi, and Texas.

In the same loblolly pine seed orchard cited by Dwinell (1976), Kuhlman et al. (1982) reported further evidence of differential clonal susceptibility. The authors grouped trees into three susceptibility classes according to the number of branches killed by pitch canker. Only 2% of the ramets in the resistant class had pitch canker in contrast to 39% in the intermediate class and 92% in the susceptible class. Susceptible clones appeared randomly located throughout the seed

orchard. When artificially inoculated, the amount of branch dieback of clones in the susceptible class was significantly greater than for clones in both the intermediate and resistant classes.

Clonal differences in pitch canker disease severity were also reported in two loblolly pine seed orchards in Alabama, although such clonal ranking varied throughout sections of the orchard (Kelly and Williams 1982). No significant differences in pathogenicity were detected from isolates of the pathogen obtained from these orchards. Significant differences in pitch canker incidence were observed among clones selected within a limited (145 km radius) geographic area in Alabama.

In a study of genetic relatedness of pitch canker susceptibility, 12 half-sib loblolly pine families from clones of various infection levels in a severely affected seed orchard were inoculated with the pitch canker fungus (Dwinell and Barrows-Broaddus 1979, 1980). Shoot mortality varied significantly among the families screened, but there was no correlation between the response of the 12 selected loblolly pine families and the shoot dieback of the parent clones. No mention was made of how the clones were evaluated.

Using inoculation techniques similar to those of Dwinell and Barrows-Broaddus (1979, 1980), Oak (1985) reported the relative ranking of susceptibility among 16 half- and full-sib loblolly pine families varied considerably between replicates based on average shoot mortality of 15 inoculated seedlings per family. The author concluded that successful inoculation of loblolly pine is more difficult using the same inoculation techniques successfully applied to slash pine, although the inoculation results of relative family resistance generally agreed with observed field performance.

Slash pine. In field studies, clones and half-sib families of slash pine differed significantly in the occurrence of natural pitch canker infections and successful infections from inoculations. Phelps and Chellman (1976) reported clonal variation in pitch canker infection based upon a severity index (a ranking of location and amount of disease within a crown) from a survey of slash pine seed orchard trees in Florida. Certain slash pine clones were heavily infected while others were apparently disease free. The apparently random occurrence of pitch canker throughout the orchards suggested that differences in infection resulted from differential host resistance.

In a more detailed evaluation of variation in susceptibility involving inoculation of shoots, 8 of 50 mature slash pine clones exhibited high levels of infections while 12 clones showed no internal or external evidence of infection (Blakeslee and Rockwood 1978). Clones originating from natural stands within central east Florida had less shoot dieback and shorter average proximal canker length than clones from either southeast Georgia or west Florida-Alabama-Mississippi regions. Evaluation of greenhouse-grown progeny from these clones was consistent with the parent performance (Blakeslee et al. 1979).

Considerable variation in the amount of successful infections among 46 half-sib slash pine families was reported by McRae et al. (1985) following inoculation with the pitch canker fungus. Host families consisted of 3-yr-old field-planted trees. Symptoms (any observable host response) by family averaged 61%, ranging from 17% to 91%, and height growth was unrelated to levels of infection.

Differences among 92 7-yr-old, half-sib slash pine families to natural infections also differed significantly (Lowerts et al. 1985). Variation in pitch canker susceptibility was closely related among families; those with select parents indigenous to Florida had significantly fewer infections than families from three

regions of Georgia. Relative ranking of susceptibility was not significantly influenced by fertilization among four check families, and growth rate in both the fertilized and unfertilized block evaluated by height and diameter, was not strongly correlated with susceptibility.

Greenhouse studies also demonstrated significant variation in susceptibility among inoculated half-sib slash pine seedling families (Blakeslee et al. 1979, Dwinell and Barrows-Broaddus 1980). Variation in mean canker length (11 mm to 77 mm) or the percentage of shoot dieback (29% to 100%) for 1.5-yr-old greenhouse-grown seedlings was reported similar as those occurring on mature trees (Blakeslee et al. 1979). The authors suggest, however, that the high level of host dieback (mean 73%) may indicate that juvenile trees do not express the same level of resistance to infection found in mature trees evaluated in a similar manner. Kraus Schmidt (1976) reported a similar relationship of host age and expression in pitch canker susceptibility for eastern white pine. Juvenile white pines were very susceptible to inoculation with the pitch canker fungus, but infection could not be induced in older trees.

Dwinell and Barrows-Broaddus (1980) cite unpublished data on an expanded version of an earlier study (Dwinell and Barrows-Broaddus 1979) that included 43 open-pollinated families of slash pine collected from trees in natural stands in Florida. Three of the families were rated as highly susceptible, 33 families as moderately susceptible, and seven families as highly resistant.

Pathogen Variability

Several studies evaluating pathogenic variability of Fusarium subglutinans suggest pathogenic specialization on members of the genus Pinus. Isolates of

Fusarium subglutinans pathogenic on plants of the genera Zea, Dracena, Lilium, Araucaria, and Amaryllis, isolates from agricultural soil, and isolates from a deodar weevil (Pissodes nemorensis Germ.) were nonpathogenic when inoculated into greenhouse-grown, 4-yr-old slash and loblolly pines (Dwinell and Nelson 1978). In comparison, isolates from pitch cankers on six pine species, loblolly pine shoots damaged by the subtropical tip moth (Rhyacionia subtropica Miller), and needle midge (Contarina spp.) lesions on slash pine were all pathogenic on pine. Fusarium subglutinans isolated from gladiolus corms was the only nonpine source consistently pathogenic on slash and loblolly pines (Barrows-Broaddus and Dwinell 1980). Conversely, different isolates of Fusarium subglutinans caused various amounts of decay when inoculated into gladiolus corms (Barrows and Dwinell 1978, Barrows-Broaddus and Dwinell 1980). Corms inoculated with isolates from other gladiolus corms, pine plantation soil, and slash pine caused the most decay and isolates from longleaf, south Florida slash, and Virginia pines produced slightly less decay. The least corm decay resulted from loblolly and shortleaf pine isolates.

Initial studies on the differences in virulence of the pitch canker fungus among different species of pines were part of an effort to taxonomically characterize the pathogen (Kuhlman et al. 1978). Nine species of Fusarium isolated from eight pine species were inoculated into 3 cm long knife wounds cut into the new shoots of 1-yr-old slash and loblolly pine seedlings. For the slash pine test, fungal cultures containing mycelium and spores were introduced into the wounds. A 0.2 ml aliquot of 1 X 10^6 conidia/ml suspension served as inoculum for the loblolly pine test. Results were evaluated on the amount of shoot dieback after eight weeks. Sixty-two of the 66 isolates from the Fusarium subglutinans group were pathogenic on slash and loblolly pine seedlings. No differences in the amount of

shoot dieback among the seven pine Fusarium subglutinans isolate sources were evident in either the slash or loblolly test seedlings. No other species of Fusarium proved pathogenic on pine.

In comparison, Dwinell (1978) reported apparent differences in virulence among six pitch canker isolates from several pine species. A Virginia pine isolate inoculated into loblolly pine seedlings produced a low percentage (16%) of shoot dieback compared to other isolate sources (mean 49%). The author indicated, however, that the inoculation procedure of slit-wounding new shoots and placing a small amount of fungus thallus into the wound was not the best for quantifying these relationships. In the same report, pathogen variation was not apparent in a companion investigation evaluating pine isolate sources and various pine host species.

Virulence was similar among 25 isolates of Fusarium subglutinans from diverse sources when evaluated on the percentage of shoot dieback of inoculated 1-yr-old loblolly and Virginia pine seedlings (Barrows-Broaddus and Dwinell 1979). Fungal isolates were obtained from pitch cankers on six species of pine, loblolly pine needles fed on by tip moth, midge lesions on slash pine needles, sporodochia of Fusarium subglutinans on slash pine, soils from seed orchards and pine plantations, and a deodar weevil pupae. Only the isolates from the plantation soil and the deodar weevil were avirulent. Blakeslee et al. (1980b) reported, however, isolates of Fusarium subglutinans from deodar weevils were pathogenic on both sand and slash pine seedlings. In addition, isolates from sand and slash pine were equally pathogenic when inoculated into seedlings of either host species.

Dwinell and Barrows-Broaddus (1980) cite unpublished data indicating a possible exception to the reported limited pathogenic variability in the pitch canker fungus. A loblolly pine isolate from South Carolina was reported virulent

on loblolly pine but did not infect slash pine. Similar racial differences in pine pathogenicity were previously suggested by Hepting (1971) to account for pitch canker infection of loblolly pine in North Carolina.

Pitch canker isolates from Virginia and shortleaf pines did not differ significantly in virulence when evaluated in 11-yr-old Virginia pine progeny (Dwinell and Barrows-Broaddus 1983). The test trees were inoculated by chisel-wounding a 3 cm^2 area of bark, exposing the cambium, and spraying the area to run-off with 1 X 10^6 conidia/ml suspension of each isolate. Main stem canker size among isolates was not significantly different after 14 mo.

Greenhouse-grown seedlings of four species of southern pines inoculated with isolates cultured from pitch cankers on loblolly pines in North Carolina and Virginia pine in Alabama also did not vary significantly in percentage of shoot mortality (Barrows-Broaddus and Dwinell 1983). In this study, bulk collections of Virginia, slash, loblolly, and pond pine seedlings were inoculated with a puncture wound through a droplet of conidial suspension. Pathogenic variation was not evident between the same pitch canker isolates for inoculated 1-yr-old half-sib Virginia pine seedlings and 11-yr-old full-sib Virginia pines. Aerial mycelium, teased from cultures, was introduced into puncture wounds on branches in the progeny test study. Percentage of shoot or branch dieback was not significantly different between the isolate sources in either study.

Influences of Soil Fertility and Host Nutrition on Tree Diseases

Influences of inorganic fertilization on forest tree diseases have been reviewed (Dimitri 1977). In most circumstances, however, the specific influence of fertilization on the host-pathogen interaction is unknown.

It is not possible to generalize on the influence of a specific nutrient among pathosystems and environments. A particular nutrient may promote some diseases while decreasing others (Huber 1980b). Bark necrosis caused by Dothichiza populea Sacc. & Briard infections on Populus L. 'Robusta' was significantly reduced with improvement of the upper soil layers by tillage and fertilization with nitrogen, phosphorus, potassium, calcium, and magnesium (Breuel 1969). In contrast, Donaubauer (1964) reported N fertilization generally increased the susceptibility of certain nursery-grown 1- and 2-yr-old poplar clones to Melampsora allii-populina Kleb., Septoria populiperda Wat., and Dothichiza populea. Nonfertilized 2-mo-old Populus tremuloides Mich. seedlings inoculated with Hypoxylon pruinatum (Klot.) Cke., however, exhibited greater canker development than plants watered with fertilizer solution (available nutrients: 7% N, 6% P, 19% K) (Bagga and Smalley 1974).

In some instances, fertilization with inorganic nutrients appears to have a greater influence on disease development than does inoculum potential (Huber and Watson 1974). The percentage of infected slash pine seedlings differed significantly between inoculation treatments using two inoculum density levels of the fusiform rust fungus (Cronartium quercuum (Berk.) Miyabe ex Shirai f.sp. fusiforme) without fertilization but was similar with fertilization (Rowan 1977a, 1977b). Rowan (1978) reported the incidence of rust infection in nonfertilized slash pine seedlings inoculated once with fusiform rust was significantly greater than several weekly inoculations with an equal cumulative spore load, but disease incidence on fertilized seedlings did not differ when subjected to the same inoculation treatments.

Host genotypes intermediate in disease resistance are generally influenced by changes in the nutritional environment while highly susceptible or resistant

genotypes are most likely to remain unaffected (Huber 1980a). Fertilization of field-grown loblolly pine increased the overall number of fusiform rust galls per infected tree (Powers and Rowan 1983). This increase was significant on susceptible seedlings, but not for seedlings from a resistant source. Hollis et al. (1977) found similar results for inoculated slash pine seedlings grown in greenhouse pot cultures. Rust incidence for a resistant half-sib family was not significantly altered by applications of either N, P, or K, but additions of both P and K to seedlings of the susceptible family significantly increased rust incidence.

Similar interactions between genetic and environmental influence on disease resistance to Pollaccia radiosa (Lib.) Bald. is also reported for poplar (Weisgerber 1968). One group of host clones showed the same response at all test sites indicating genetic control of resistance is so strong that edaphic influences are inconsequential. Resistance in the other genotype group varied substantially among test sites.

Increases in disease severity often occur when the level of a particular nutrient is sufficient, but the balance and/or nutrient form is altered (especially evident among forms of available N) (Huber 1980a). Severely nutrient stressed plants, either in deficit or excess, are generally more predisposed to disease. Infection of leaves of a fast growing poplar clone by Marssonina brunnea (Ell. et Ev.) Sacc. was limited if nutrition was adequately balanced and sufficient (Garbaye and Pinon 1973). Severe leaf damage from infection occurred if any one anion (NO_3, H_2PO_4, SO_4) was lacking and insufficient Mg or Mn resulted in greater leaf chlorosis and increase in susceptibility. Heavy ground applications of NPK fertilizer plus micronutrients reduced the intensity of Ohia decline in Metrosideros collina (Forst.) Gray subsp. polymorpha (Gaug.) Rock stands in Hawaii. Declining trees responded only to the complete fertilizer or a combination

of N and P, not to applications of any single macronutrient or mixtures of micronutrients (Kliejunas and Ko 1974).

Host Defense Mechanisms Influenced by Nutrition

Although not clearly understood, several nutrient specific host defense mechanisms are reported to be involved in host-pathogen-nutrient interactions (Huber 1980b). These mechanisms are 1) disease tolerance through nutritional compensation for pathogenic damage, 2) phenological disease escape via. changes in host tissue maturation, 3) physiological resistance by changes in the trophic environment of the pathogen, or 4) alterations in pathogen virulence.

Disease tolerance. Disease tolerance can be influenced by fertilization through compensation for limited uptake from the loss of absorptive tissues. Nutrient deficiency symptoms and decline of littleleaf diseased (Phytophthora cinnamomi Rands) shortleaf pine arise from root system deterioration from attack of the rootlets of low vigor trees on poor sites (Mistretta 1984). Compensatory applications of N prevented disease onset or reversed early symptom development, including reduced growth, in infected shortleaf pine (Roth et al. 1948). A similar compensatory effect of fertilization is reported for Scots pine infected with Armillaria mellea (Vahl.) Karst. (Rykowski 1981). Fertilization of naturally infected plantations with NPKCaMg reversed, maintained, or prevented the appearance of crown symptoms even if the roots were partially colonized by the pathogen. The number of attacked trees, however, was independent of fertility treatment.

Phenological disease escape. Huber (1980b) defines five possible mechanisms of phenological disease escape by plants based on host tissue development

that could be altered through fertilization. These include 1) reducing the infective period by shortening the susceptible vegetative period, 2) outgrowing the pathogen, 3) hastening of wound healing, 4) strengthening cell walls and thickening cuticle layers impeding pathogen penetration, and 5) hastening of periderm formation. Literature on this subject is sparse concerning tree diseases.

Host vigor in itself is not considered a criterion for susceptibility or resistance (Huber 1980b). Fertilization with different forms of N can result in minimal differences in succulence; however, one form may enhance susceptibility and another resistance (Huber and Watson 1974).

Physiological resistance. Altered host nutrition is reported to affect physiological resistance by 1) influencing nutritional compatibility with the pathogen through modification of the nutritional environment, and 2) inhibition of pathogenic activity through the accumulation of inhibitory compounds around infection sites (Huber 1980b).

Clones of several poplar hybrids showed higher resistance against Melampsora larici-populina Kleb. when grown in solution cultures deficient in N and P (Suzuki 1973, Suzuki and Chiba 1973). The resulting reduced sugar content in the leaves was considered an important resistance factor.

Compounds that change in conifer tissues in response to N fertilization include free amino acids and the wide variety of derived secondary products (Durzan and Steward 1967). Among N sources, increased availability of ammonium over nitrate leads to an abundance of specific free amino acids (predominantly arginine) and other derivatives (e.g., monosubstituted guanidine compounds) (Durzan and Steward 1983). Certain monosubstituted guanidine compounds provide excellent N sources for fungi colonizing dead plant tissues and fallen timber, while others are antibiotic (e.g., streptomycin). Enhanced

accumulation of these compounds following excessive application of nitrogenous fertilizers may negatively affect tree growth (Durzan 1974). Reviewing the effects of N fertilization on plant disease in general, Huber and Watson (1974) reported form (e.g., ammonium, nitrate, etc.), rather than availability, was most the influential factor on disease severity.

Infection by Diplodia pinea (Desm.) Kickx. was associated with sulfur deficiencies in Pinus radiata (Lambert and Turner 1977). Limited soil sulfur levels resulted in deficiencies of sulfur-containing amino acids in the host, reduced tree growth, and lead to the accumulation of other free amino acids. The authors conclude this type of nutrient deficiency resulted in a metabolic dysfunction promoting apical resinosis, allowing an entry point for the fungus into the shoot, and building up of particular amino acids (esp. arginine) that improved trophic conditions for the invading fungus.

Pathogen virulence. Host nutrition can influence the pathogenicity and virulence of a pathogen in addition to those aspects of host disease resistance previously mentioned (Huber 1980b). Pathogenicity and virulence factors directly influenced by the nutritional status of the host include propagule germination, growth, penetration, and/or enzymatic activity. Factors indirectly affected are fungistasis or survival.

Increases in the virulence of Phomopsis juniperovora Hahn were reported via. stimulation of pathogen germination and pathogenesis by leachate rich in K and unsaturated carboxylic acid for susceptible eastern red cedars (Juniperus virginiana L.) (Pero and Howard 1970).

The composition of cuticular waxes, greatly affected by environmental factors, significantly influenced fungal infection by Lophodermium pinastri (Schard. ex Hook.) Chev., Botryis cinerea Pers. ex Fr., Rhytisma acernum Pers. ex Fr.,

Microsphaera alphitoides DC., and Fusarium oxysporum (Schl.) em Snyd. & Hans. by favoring or retarding mycelial growth (Schutt 1971, 1972).

In culture, colony development of Armillaria mellea relating to pathogenicity was greatly influenced by the concentration of carbon (D-glucose) and nitrogen (L-asparagine) in the media (Rykowski 1976). Carbon tended to favor production of aerial mycelium and the transformation of rhizomorphs from the infectious subterranea type to the assimilatory subcorticalis type. Conversely, high N concentrations inhibited mycelium production and stimulated rhizomorphogenesis.

Pathogenesis relating to fungal metabolites may also be altered by nutritional conditions of the host. Synthesis of oxalic acid by Heterobasidion annosum (Fr.) Bref., secreted when growing inside the living tree, was inhibited in artificial cultures by the addition of L-threonine to the growth media (Huttermann et al. 1980). Inhibition of the same biosynthetic pathway in Sclerotium rolfsii Sacc. diminished pathogenesis on lima bean seedlings (Kritzman et al. 1977).

Associations of Soil Fertility and Host Nutrition with Pitch Canker

Field observations and experimental evidence suggest strong relationships among soil fertility, host nutrition, and pitch canker incidence. In most instances, increased N fertility, alone or in combination with P and/or K amendments, has been associated with increases in pitch canker incidence and severity (Cashion 1979, Cleason 1978, Cleason and Smith 1978, Fisher et al. 1981, Fraedrich 1979, Fraedrich and Witcher 1982, Lowerts et al. 1985, Wilkinson et al. 1977).

In 1966, personnel from the Florida Division of Forestry reported severe pitch canker infection and associated tree mortality in a slash pine plantation

immediately adjacent to a poultry farm near Waldo, Florida (cited in: Wilkinson et al. 1977). Cleason and Smith (1978) also reported an increasing gradient of pitch canker incidence in a 9-yr-old slash pine plantation coincided with increasing levels of foliar nutrients and soil fertility from a nearby poultry operation. Levels of N, P, and K in the foliage of the infected slash pine were highest adjacent to the farm but fell to within nominal values a short distance away. Aerial concentrations of N and P followed a similar pattern. Soil levels of P were higher near the farm while N and soil reactivity remained unchanging with distance.

An unusually high incidence of pitch canker in a 19-yr-old slash pine seed production area in Flagler County, Florida, during 1968 was associated with chemical fertilization (cited in: Wilkinson et al. 1977). Four years earlier the trees had been thinned to 100 stems/ac (247 stems/ha) and fertilized with 7 lbs (3.18 kg) N/tree/year. An analysis of environmental factors from a survey of seed orchards and plantations in Florida during a major pitch canker outbreak of the mid-1970s indicated disease incidence and damage were greatest on flat, sandy, wet sites that were fertilized and burned sometime during the stand history (Phelps and Chellman 1976).

Mineral foliar content was not useful in identifying levels of susceptibility (based on the number of branches killed in a crown) among seed orchard loblolly pine clones during a disease outbreak (Kuhlman et al. 1982). Foliage of clones with intermediate susceptibility were significantly greater in concentrations of K, NH_4, and B; susceptible clones had significantly greater foliar Ca amounts, but these differences were all within normal levels for loblolly pine.

In addition to field observations of pitch canker outbreaks, experimental evidence examining the interaction between fertility and pitch canker indicates a positive correlation between increased host susceptibility (generic for increases in

incidence and severity) and N fertility, with or without P and/or other nutrients. In most of the field studies, the influence of fertilization on pitch canker disease was determined by observations of naturally occurring infections.

Increased frequency of pitch canker in planted slash pine was consistently associated with applications of high levels of N (Wilkinson et al. 1977). The following treatments were applied each year for six consecutive years to plots containing 40 seedlings planted adjacent to a slash pine seed production area that was heavily infected with the pitch canker fungus: 1) 200 lbs N, 200 lbs P, and 200 lbs K/ac (224.16 kg/ha), 2) no fertilization, 3) 200 lbs N/ac and, 4) 200 lbs P plus 200 lbs K/ac. After the first growing season, both of the high N treatments had the highest pitch canker incidence (mean 50%). After six years of fertilization, the highest incidence of pitch canker (69%) occurred in the complete fertilizer treatment plot, with the other treatments ranging from 26% to 40% infection.

Application of urea (200 and 400 lbs N/ac [224.16 and 448.32 kg/ha]) plus P (100 lbs P/ac [112.08 kg/ha]), as super concentrated phosphate, to a pitch canker-infected 12-yr-old slash pine stand growing on a spodosol in east-central Florida resulted in increased tree mortality and reduced diameter growth (Fisher et al. 1981). These N and P treatment effects were counteracted, however, when K, Ca, Mg, and fritted micronutrients were also applied. Lesser amounts of N and P (0, 100 [112.08 kg/ha], and 0 lbs/ac, respectively) or additions of either element alone did not significantly affect disease severity.

Levels of naturally occurring pitch canker infection in slash pine plantations on a variety of soil types in west-central Florida were not significantly influenced by applications of 36 lbs N/ac (40.35 kg/ha) and 40 lbs P/ac (44.38 kg/ha) as diammonium phosphate (Anderson and Blakeslee 1984, Blakeslee, personal communication). Foliar tissue analysis of fertilized stands showed P

levels equal to, or greater than, the unfertilized plots. Pitch canker incidence averaged 28% in the unfertilized plots compared to 30% for the fertilized plots. Comparisons of disease severity and incidence between treatment plots in previous years also showed no trends. The authors suggested that fertilization of young established slash pine stands with low N did not adversely influence pitch canker.

Fertilization at the time of planting (45 lbs N/ac [50.44 kg/ha] and 50 lbs P/ac [56.04 kg/ha]) of open-pollinated slash pine families significantly increased the number of naturally infected trees after seven growing seasons (Lowerts et al. 1985). Treatments were administered to 92 families planted on a spodosol in an area of Volusia County, Florida, with a history of extensive pitch canker damage. Only four families were common to both the fertilized and untreated plots; however, among all families, 40% of the trees were infected in the fertilized plot while only 20% were infected in the check plot. Family effects and fertilizer x family interactions were not significant among the four check families.

In a greenhouse pot study, high levels of N from a combined source of ammonium nitrate and ammonium chloride, regardless of P and K levels, were associated with greater amounts of shoot mortality and fascicle necrosis of inoculated slash pine seedlings (Cleason 1978). All possible combinations of two levels of N, P, and K (20 and 100, 4 and 20, and 15 and 80 ppm, respectively) were applied in a factorial arrangement to 10 half-sib slash pine seedlings potted in acid washed sand. All seedlings were given the same amounts of other nutrients. Four months after receiving the soil nutrient treatments, the seedling shoots were puncture wounded and inoculated with an 1×10^6 conidia/ml aqueous suspension. Pitch canker susceptibility evaluated as symptoms of resinosis, fascicle necrosis, or amount of shoot mortality was not influenced by the P and K treatments. Average days to shoot death and average length of stem discoloration were also

not significantly influenced by any nutrient treatment. Nitrogen concentration of shoot tissues was higher in seedlings expressing symptoms of shoot mortality and fascicle death while contents of Ca and Mg were significantly lower. Concentrations of P and K did not vary between alive or dead shoots. A significantly lower pH was associated with the potting media of symptomatic seedlings.

Increasing N fertilization significantly increased the number of dead shoots from inoculations of 1-yr-old slash pine seedlings (Cashion 1979). Flats of sand cultures containing the seedlings were treated with applications of 60 and 120 ppm N as ammonium nitrate. The N treatments were divided among four levels of P at 0, 30, 45, and 60 ppm. Nutrients were applied 8 wks before and after inoculation. Seedlings were inoculated with aqueous suspensions of 1 X 10^5 conidia/ml introduced through a needle puncture on the succulent portion of a terminal stem. Phosphorus effects and N x P interactions were not significant in shoot mortality response.

In a series of seven related greenhouse and field studies, Fraedrich (1979) and Fraedrich and Witcher (1982) associated N fertilization, usually in combination with P and K, with increases in mean canker length of inoculated slash, Virginia, and loblolly pine seedlings and field-grown trees. Field studies varied according to inoculation procedures (PDA disc or spore suspension into chisel wounds to the lower main stem or terminal), inoculation dates (31 Mar to 20 Aug), intervals between fertilization and inoculation (1 to 8 wk), inoculation to symptom evaluation periods (4 to 7 mo), fertilization rates (205 to 503 lbs/ac N [229.76 to 563.76 kg/ha], 89 to 236 lbs/ac P [99.75 to 264.50 kg/ha], and 170 to 418 lbs/ac K [190.54 to 468.49 kg/ha]), and plot characteristics (Piedmont and sandhills). The same inoculation procedures (PDA disc into cork borer wound at tree base), intervals between fertilization and inoculation (4 mo), inoculation to

symptom evaluation time (3 wk), and fertilization rates (0.177 gm/6 L pot of N, P, and K biweekly) were used in all of the greenhouse studies but inoculation dates (15 Jan to 13 Sep) and seedling ages (12 to 24 mo) differed among the studies. Ammonium nitrate served as the N source for both the field and greenhouse studies.

Fertilization with N in various treatment combinations with other elements significantly increased mean canker length among the greenhouse experiments. Mean canker length (7.6 cm) on 12-mo-old greenhouse-grown slash pine seedlings was significantly greater only when fertilized with a combination of NPK after 21 days. In 24-mo-old slash pine seedlings, however, any N treatment alone or in combination with other elements resulted in significantly larger mean canker length (4.7 cm) than the control (2.4 cm). Canker lengths of seedlings treated with P, K, and PK were not different from the control. Virginia pine seedlings fertilized with N alone or in combination with the other elements had significantly larger cankers (4.5 to 5.3 cm) than all other treatments (2.7 cm to 3.1 cm). Cankers did not vary among N treatments or among treatments where N was absent. Mean canker length (4.7 cm) was also significantly greater on greenhouse-grown loblolly pine seedlings receiving the N and NPK treatments.

Response to fertilization treatments was significant in two of the four related field studies reported by Fraedrich (1979) and Fraedrich and Witcher (1982). In both of these studies fertilization with NPK resulted in longer average canker lengths. Average canker lengths of slash pine growing in the Piedmont and fertilized with NPK were significantly longer than on non-fertilized trees 7 mo after inoculation (21.2 cm vs. 13.9 cm, respectively). On 8-yr-old slash pine growing in the sandhills region, differences in mean canker length were not

statistically significant (5.5 cm to 7.6 cm) among fertilizer treatments consisting of N, P, and K alone, in all possible combinations, and no fertilizer treatment.

Average canker length from inoculations of terminal shoots did not significantly differ among fertilizer treatments consisting of separate applications of N, P, K, a combination of all three, and nonfertilized (5.0, 4.9, 4.7, 5.1, and 4.8 cm, respectively) on 6-yr-old loblolly pine. When the boles of the same trees were inoculated for another study a year later, however, mean canker length was significantly greater (14.1 cm) on those trees fertilized with the combination of nutrients than in the other treatments.

CHAPTER III

INFLUENCES OF PATHOGEN VARIABILITY, INOCULUM CONCENTRATION, WOUNDING TECHNIQUE, AND SEASON OF INOCULATION ON DISEASE EXPRESSION OF SLASH PINE CLONES INOCULATED WITH THE PITCH CANKER FUNGUS

Introduction

The development of pitch canker disease is regulated by genetic variability in pathogen virulence and host susceptibility and by numerous environmental and epidemiological factors such as season of year when exposed, type of wound, and inoculum concentration. Knowledge of the contributions and interactions of these factors in disease expression would provide useful insight into the disease biology of pitch canker. This information would define appropriate inoculation technologies for host-pathogen-environment interaction studies, explain past disease outbreaks, and identify potential forest pest management strategies.

Differences in virulence among pine pathogenic strains of Fusarium subglutinans may be important in disease expression. Differences in amounts of shoot dieback were not significant among Fusarium subglutinans isolates obtained from soil, insects, or various species of pine evaluated using several different species of pine hosts (Barrows-Broaddus and Dwinell 1979, 1983, 1984, Dwinell and Barrows-Broaddus 1983). Significant differences in shoot dieback were reported, however, among pitch canker isolates recovered from branches, cones and seeds of loblolly pine but only when tested with Virginia pine seedlings and not loblolly pine seedlings (Barrows-Broaddus and Dwinell 1985). A notable exception to the apparent limited variation of the pitch canker fungus may be an isolate

from loblolly pine in South Carolina reported highly aggressive on loblolly pine but avirulent on slash pine (Dwinell and Barrows-Broaddus 1980). Differences in pitch canker disease expression in slash pine stands, and within the same stand over time, indicates geographic and temporal variation in pathogen populations may exist among diverse regions within Florida.

Variation in susceptibility of slash pine to pitch canker was demonstrated in several studies among clones, full- and half-sib families (Blakeslee and Rockwood 1978, Blakeslee et al. 1979, Dwinell and Barrows-Broaddus 1980, Rockwood et al. 1988). Heritability estimates for resistance to pitch canker suggest selection and breeding would substantially decrease disease incidence (Rockwood et al. 1988). Inoculation of the host tree insures against disease escapes, a concern present in studies relying on natural infection by wound-requiring pathogens such as the pitch canker fungus. Furthermore, genetic expression of pitch canker susceptibility in slash pine may be modified by inoculation techniques in addition to other epidemiological and environmental factors.

A number of inoculation techniques and dosages of inoculum were previously used to evaluate host susceptibility to the pitch canker fungus. Knife slit wounds through the bark into the xylem of shoots were inoculated with fungal cultures (Dwinell 1978, Hepting and Roth 1946) and aqueous conidial suspensions (Kuhlman et al. 1982). Aqueous conidial suspensions of varying concentrations and quantities were used to inoculate chisel wounds into the boles of mature trees (Dwinell and Barrows-Broaddus 1983, Fraedrich and Witcher 1982), wounds in seedlings made by removal of needle fascicles (Kelly and Williams 1982), and small puncture wounds made through the outer bark of juvenile and mature host trees (Barrows-Broaddus and Dwinell 1984, Blakeslee et al. 1978, Kuhlman et al.

1982). Needle puncture wounds were also inoculated with aerial mycelium from pitch canker fungus cultures (Barrows-Broaddus and Dwinell 1983).

In comparing wounding techniques, no differences in the amount of shoot dieback of loblolly pine were reported between bark slitting and needle puncture wounds inoculated with conidial suspensions (Kuhlman 1987, Kuhlman et al. 1982). Oak (1985), however, reported that the puncture wounding technique successful for slash pine produced inconsistent results when inoculating loblolly pine.

Pitch canker expression in slash pine may also be sensitive to inoculum concentration. Kuhlman (1987) reported inoculations with increasing inoculum concentrations resulted in increased amounts of shoot dieback of loblolly pine seedlings. Defining the effects of inoculation procedures involving wounding technique and inoculum concentration could provide standard practices for estimating and comparing levels of pitch canker resistance and aid in the interpretation of study results.

Pitch canker expression may also be influenced by the season of year during which the tree is exposed to the pathogen. In loblolly pine, Kuhlman et al. (1982) reported fall and winter inoculations produced greater amounts of branch mortality than spring and summer inoculations. Although slash pine is susceptible (able to be infected) throughout the entire growing season, the preponderance of naturally occurring pitch canker infections occur in the late summer and fall in Florida (G. M. Blakeslee and S. W. Oak, unpublished).

Tree age may also affect pitch canker expression. Phelps and Chellman (1976) reported the highest incidence of pitch canker infection in plantation-grown slash pine occurred in age classes of 17 years and older. The highest levels of mortality attributable to pitch canker infection was observed in a 23-yr-old slash

pine plantation (Blakeslee and Oak 1979). Furthermore, reports of major epiphytotics in young slash pine stands are rare. Evaluation of older slash pine trees may be important for estimating field performance of pitch canker susceptibility.

Host responses other than shoot or branch dieback may be beneficial in evaluating pitch canker disease resistance. Blakeslee and Rockwood (1978) related average proximal canker length of inoculated branches to the proportion of branch dieback among clones. Hypertrophic callus-like tissue at the inoculation site was observed for slash and other susceptible pine hosts in the absence of diffuse canker development and branch dieback symptoms (Barrows-Broaddus and Dwinell 1984, Fraedrich and Witcher 1982). These various responses may be phenotypic expressions of genotypic host differences representing resistance mechanisms. Their quantification could provide additional selection criteria in breeding for pitch canker resistance.

Few studies have examined the effects of inoculum concentration of various inoculum sources, the possible effects of seasonal changes in host susceptibility, or the nature of the inoculation wound on different response reactions among slash pine host genotypes. The objectives of this study were to determine the influences and interactions of selected host, pathogen, environmental, and inoculation technique factors on the expression of pitch canker disease in mature field-grown slash pines. Results of this study will be useful in identifying inoculation methodologies and host resistance responses for use in evaluating fertilization influences in Chapter 4 and for use in selection and breeding for pitch canker resistance. These objectives were accomplished by 1) comparing the relative susceptibility of clonal slash pine genotypes through disease survey and inoculation procedures, 2) examining seasonal influences on host response, 3) evaluating selected host

responses as expressions of inherent resistance, 4) evaluating differences in virulence among selected geographic and temporal inoculum sources, 5) determining the effects of inoculum concentration on disease expression, and 6) identifying consistent and reliable inoculation techniques including preinoculation branch surface sterilization procedures.

Materials and Methods

Branch Surface Sterilization

Host material. Healthy, first-flush shoots from the current growing season were collected in July 1982, from 15-yr-old, planted slash pines located in Gilchrist County, Florida. Pitch canker infection was evident in the stand but branches were collected from disease-free trees. Careful collection and handling of the branches limited contamination or disturbance of the microbe population resident on the branch surface.

Sterilization treatments. Ethanol at concentrations (v/v) of 50%, 70%, and 95%, and hydrogen peroxide at 5%, 15%, and 30%, were used as surface sterilants. For each treatment, five branch sections were moistened to runoff. Five sections were untreated as controls for each treatment.

Following treatment, the branches were air dried, the needle fascicles were aseptically removed, and the branch sections were rolled across the surface of a Fusaria selective PCNB media (Nash and Snyder 1962) in 11 cm petri dishes and the dishes were incubated at 24 C with a 12 hr photoperiod. After 10 days, cultures developing on the agar surface were examined to determine the number of colonies of Fusarium spp. present. The most effective treatment in reducing

surface residing Fusaria would be used for surface sterilization of branches prior to inoculation in the following studies.

Inoculation Techniques Among Host Genotypes and Fungal Isolates

The influence of five potential response-regulating variables (slash pine genotype, pitch canker inoculum source, inoculum concentration, wounding technique, and season of inoculation) on pitch canker disease expression were examined in two experiments. Because available host material was insufficient to address all five variables in a single comprehensive test, related variables were divided among the two experiments. Three types of host responses were evaluated as 1) branch dieback, 2) callus formation, and 3) canker length.

Experiment I

The effects of four sources of the pathogen at three inoculum concentrations on disease expression was evaluated among four selected slash pine clones. Test branches were wounded by the puncture wounding technique during the summer season.

Host genotype. Host genotype influences were evaluated among eight ramets for each of four 20-yr-old slash pine clones growing in a seed orchard on a uniform site in Bradford County, Florida (Appendix). The host clones represented various degrees of apparent pitch canker susceptibility based on current and historical evidence of naturally occurring pitch canker infections when evaluated in August 1982. Ramets of two clones had evidence of both current and previous infection, one clone had only historical evidence of infection, and one clone had no

evidence of pitch canker infection. The four host clones were again evaluated for naturally occurring pitch canker infections 8 mo later in May 1983, at the conclusion of the study. During this second evaluation, each ramet was scored on the proportion of crown infected a) <10% = 0, b) 10-30% = 1, c) 30-50% = 2, d) 50-70% = 3, and e) >70% = 4.

The inoculation tests utilized tissues of the second flush of the year (first summer flush) of vigorously growing branches randomly selected from within the upper crown of each ramet. These branches were chosen for uniformity of phenology and physical condition, viz. free of obvious injury and without conelets.

Inoculum source. To assess variation in pathogen virulence, four groups of five Fusarium subglutinans isolates were obtained from natural infections on slash pines in stands throughout Florida. The stands represented degrees of disease intensity. In 1982, inoculum sources were collected from 1) a 15-yr-old plantation in Gilchrist County, Florida, characterized by occasional tree infections with light crown damage (G), 2) a 10-yr-old plantation in Franklin County, Florida, with small areas of moderately severe outbreaks (B), and 3) a 10-yr-old plantation in Volusia County, Florida, with scattered infections of light severity (NV). The fourth source (OV), was comprised of isolates from the University of Florida stock culture collection obtained from the same area in Volusia County, Florida, during a major disease outbreak in 1977.

Isolations, made from active cankers collected from the various regions, were cultured on PCNB media and incubated at 24 C and a 12 hr photoperiod for 10-14 days. Colonies appearing as Fusarium subglutinans were transferred to carnation water agar media (CWA) and grown under the same conditions to facilitate identification. Selected isolates were single-spored, their identity confirmed, and their pathogenicity to slash pines verified by inoculating small

puncture wounds on shoots of 18-mo-old slash pine seedlings with a droplet (ca. 0.03 ml) of 1 X 10^6 conidia/ml aqueous suspension. Isolates were considered pathogenic if characteristic pitch canker symptoms were observed and Fusarium subglutinans was isolated proximal to the inoculation site.

The stock culture isolates obtained in 1977 from the Volusia County, Florida, epiphytotic area were maintained on potato dextrose agar media (PDA) in culture tubes at 1 C in the dark. Selected isolates from this collection were cultured on CWA and inoculated into 1-yr-old slash pine seedlings following the same procedures for pathogenicity verification. Isolations were made from the proximal extension of lethal cankers. Recovered isolates were again single-spored, identified, and their pathogenicity verified prior to use as an inoculum source.

Inoculum concentration. The four inoculum sources were evaluated at three dosage levels (1 X 10^2, 1 X 10^4, and 1 X 10^6 conidia/ml). Monoconidial isolates were grown on CWA for 10-12 days at 24 C and a 12 hr photoperiod. Conidia from these cultures were added to sterile deionized water and concentrations were adjusted to 1 X 10^6 conidia/ml with a hemocytometer. Approximately equal proportions of macro- and microconidia were obtained by selectively scraping sporodochia and aerial mycelium, respectively. Aliquots of inoculum from the five isolates for each source were combined, yielding a polymix with equal numbers of conidia from the five isolates. Lower inoculum concentrations were prepared by diluting the highest inoculum concentration suspension to 1 X 10^4 and 1 X 10^2 conidia/ml. After each inoculation day, all inoculum was evaluated for germination and pathogenicity.

Experiment II

Three techniques of wounding were tested during two different seasons of the year using the same eight ramets of the same four host clones. The inoculum consisted of the recent Volusia County, Florida, source (NV) at an intermediate concentration level (1 X 10^5 conidia/ml).

Wounding technique. The three wounding techniques used for inoculation consisted of 1) puncture wounds created with a syringe needle on opposite sides of the test branch in the middle of the flush and a droplet (ca. 0.03 ml) of inoculum dispensed into each wound, 2) wounds made by removal of three needle fascicles from the upper surface in the middle of the flush of the test branch and ca. 0.03 ml aliquots of inoculum placed onto each wound, and 3) nonwounded branches inoculated by placing several droplets of inoculum on the branch surface in the axis of needle fascicles located in the middle of the flush. A nonwounded, noninoculated branch was designated as a control in each ramet to assess ambient infection. Prior to inoculation, branch surfaces were misted until runoff with 95% ethanol and air dried.

Season of inoculation. The three wounding techniques were evaluated during two periods of the same growing season to examine the influence of changes in host phenology and climate. Summer inoculations were performed in a random sequence (tree by tree basis) from 22 Sep 1982 to 7 Oct 1982. During this period, active shoot growth was occurring and the summertime weather pattern of hot humid days and warm nights persisted (33 C max. and 13 C min. 24 hrs before and after inoculation).

Fall inoculations occurred on 12-13 Nov 1982. Shoot growth had ceased and fall weather patterns of warm days and cool nights had occurred (23 C max. and 6 C min. 24 hrs before and after inoculation).

Evaluation of Host Response to Inoculation

Test branches were harvested on 25 May 1983, 8 mo after the first inoculations. The branches were evaluated for symptoms of dieback, callus formation, and canker length.

Callus formation was determined by the presence of a darkened margin of hypertrophic callus tissue surrounding the inoculation site. Callus formation was recorded for branches with responses greater than those occurring from mechanical wounding alone.

Canker length was measured from the point of inoculation to the proximal extension of necrosis in the inner bark. In slash pine, pathogen invasion is commonly arrested at the branch nodes. To compensate for possible interference of the branch node in fungal invasion of host tissue, the length from the inoculation site to the proximal branch node was measured to evaluate canker length as a proportion of available tissue.

To verify Fusarium subglutinans in inoculated test branches, isolations were made from all treated branches of two ramets in each clone, and from all symptomatic branches in the other ramets. Branch tissue samples were taken from above (distal), within, and below (proximal to) the inoculation site, and at the canker margin when appropriate, and were surfaced sterilized by dipping into 95% ethanol and flaming before culturing on PCNB media for 14 days at 24 C and a 12 hr photoperiod. Fusarium subglutinans appearing colonies were

transferred to CWA for morphological identification and the pathogenicity of one isolate per branch was tested. The following priority was used to select isolates from each branch in pathogenicity testing: 1) canker margin, 2) proximal branch section, 3) distal branch section, and 4) inoculation site.

Statistical analysis. Treatment effects on branch dieback and callus formation were evaluated separately as binomial variables. Treatment effects on host response were evaluated using analysis of variance as implemented in the GLM procedure in SAS (1985). Canker length was analyzed as total proximal canker length and as a proportion of branch length (transformed as arcsin square root of the proportion of the branch). Treatments with significant interactions were analyzed separately by each level of the interacting effect. Differences among statistically significant treatment means were evaluated using least significant difference procedures (Cramer and Walker 1982).

Host clone, inoculum source, inoculum concentration, wounding technique, and season of inoculation treatments were evaluated as fixed effects. Ramets within host clone were considered as random effects.

Results

Branch Surface Sterilization

The untreated control branches yielded 16 surface residing Fusarium colonies per branch (Figure 3.1). Increasing concentrations of hydrogen peroxide reduced but did not eliminate Fusaria even at the highest concentration. Surface Fusaria were eliminated with 70% and 95% ethanol treatments. Because of its

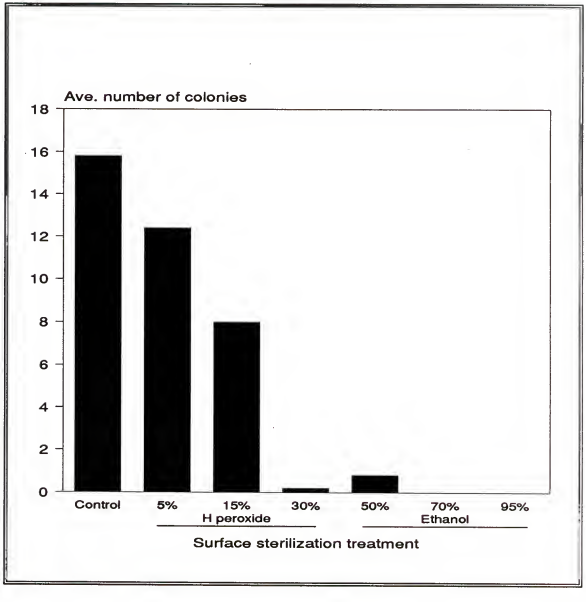

Figure 3.1. Average number of Fusarium colonies cultured from the surface of five slash pine branches following surface sterilization treatments using three concentrations of ethanol and hydrogen peroxide.

Table 3.1. Relative estimates of pitch canker susceptibility among slash pine clones from seed orchard disease surveys and artificial inoculation experiments.

Clone	1982 Survey			1983 Survey	Inoculation	
	Historical[a]	1982[a]	Est. susc.[b]	Crown infect. index[c]	Exp. I	Exp. II
					% Branch dieback[d]	
116	+	+	Susc.	2.5	57.3 a	47.9 a
122	+	-	Intr.	0.0	8.3 c	16.7 b
205	+	+	Susc.	1.5	30.2 b	6.3 bc
210	-	-	Res.	0.3	0.0 c	0.0 c

[a]Symptoms of pitch canker crown infection: + = present, - = absent.

[b]Estimated pitch canker susceptibility based on infection occurrence: Susceptible (Susc.) = historical and current infection, intermediate (Intr.) = only historical or current infection, resistant (Res.) = no evidence of pitch canker infection.

[c]Crown infection index of ramets based on level of pitch canker infection: <10% = 0, 10-30% = 1, 30-50% = 2, 50-70% = 3, and >70% = 4.

[d]Means with the same letter are not significantly different (p=0.05).

effectiveness and short drying time, the 95% ethanol treatment was used for sterilization of the branch surface.

Inoculation Techniques Among Host Genotypes and Fungal Isolates

Host genotype. Estimates of pitch canker susceptibility among host clones were closely related between the 1982 and 1983 disease surveys (Table 3.1).

Clones 116 and 205 with both historical and current evidence of pitch canker attack also had the two highest crown infection indices (2.5 and 1.5, respectively). Results from both surveys also agreed on low susceptibility estimates for clones 122 and 210.

Host clone susceptibility estimates from both surveys also were closely related to the ranking of branch dieback from inoculation experiments. Clone 116, judged most susceptible and with the highest crown infection index, was highest in the percentage of branch dieback in both experiments (57.3% and 47.9%). Clone 210, estimated as resistant in both surveys, had no branch dieback resulting from 96 inoculations in the first experiment and from 48 inoculations in the second. Survey susceptibility estimates were also related to the amount of branch dieback in Experiments I and II for clones 122 and 205.

Host clones differed significantly in the percentage of branch dieback in both inoculation experiments ($p=0.0001$ for both) (Figures 3.2 and 3.3, and Tables 3.2 and 3.3). Clone 116 had significantly greater amounts of branch dieback in both Experiments I and II (57.3% and 47.9%, respectively). In Experiment I, the percentage of branch dieback for clone 205 (30.2%) was significantly higher than 122 (8.3%). Although the ranking of the percentage of branch dieback was reversed between clones 205 (6.3%) and 122 (16.7%) in Experiment II, these differences were not significant. The change in ranking between these clones was the result of an increase in the amount of branch dieback in Experiment I from a significant clone x inoculum source interaction for clone 205 and inoculum source G. Only source NV was used in Experiment II.

The percentage of branch dieback was relatively consistent among ramets for clones 116, 122, and 210 in both inoculation experiments (Figures 3.4 and 3.5). In Experiment I, the percentage of branch dieback among ramets appeared

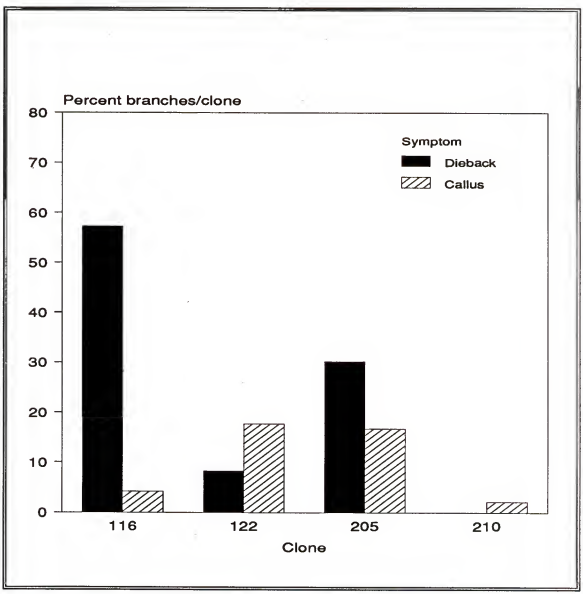

Figure 3.2. Branch dieback and callus formation of four slash pine clones inoculated with the pitch canker fungus in Experiment I.

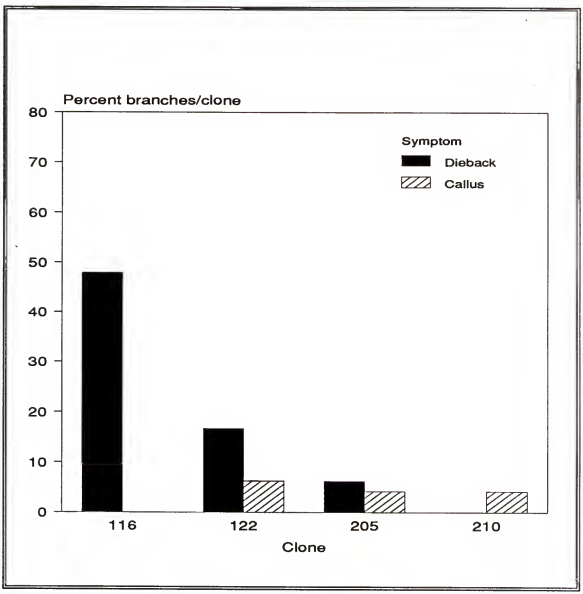

Figure 3.3. Branch dieback and callus formation of four slash pine clones inoculated with the pitch canker fungus in Experiment II.

Table 3.2. Effects of slash pine clone, inoculum source, and inoculum concentration on branch dieback following inoculation with the pitch canker fungus.

Analysis of variance				
Source	df	SS	F value	Pr. > F
Clone	3	18.90	57.59	0.0001
Inoculum source	3	0.48	1.46	0.2252
Inoculum concen.	2	3.65	16.67	0.0001
Clone*Source	9	3.83	3.89	0.0001
Clone*Concen.	6	4.98	7.59	0.0001
Concen.*Source	6	0.40	0.60	0.7278
Clone*Src.*Conc.	18	0.98	0.50	0.9587
Error	336	36.75		

variable only for clone 205. Comparing ramets in Experiment I, branch dieback ranged from 41.6% to 75.0% in clone 116, 0.0% to 25.0% in 122, and 8.3% to 75.0% in 205, and no branch dieback in clone 210. A similar pattern was observed in Experiment II. Branch dieback among ramets ranged from 33.3% to 66.7% in clone 116, 0.0% to 33.3% in 122, and 0.0% to 16.7% in 205.

Relative amounts of branch dieback response to inoculation were not associated with geographic origin of host clone. Inoculations of clones 205 and 210, both originating from north central Florida (Bradford County), resulted in moderately high levels of branch dieback and no branch dieback, respectively. The two clones of intermediate and susceptible response originated from adjacent counties

Table 3.3. Effects of slash pine clone, wounding technique, and season of inoculation on branch dieback following inoculation with the pitch canker fungus.

Analysis of variance				
Source	df	SS	F value	Pr. > F
Clone	3	6.52	25.18	0.0001
Wounding technique	2	2.82	16.35	0.0001
Season of inoc.	1	0.00	0.00	1.0000
Clone*Tech.	6	2.14	4.12	0.0007
Clone*Season	3	0.54	2.09	0.1032
Tech.*Season	2	0.59	3.44	0.0344
Clone*Tech.*Seasn.	6	0.87	1.67	0.1313
Error	168	14.50		

in north coastal Florida (Nassau and Duval Counties for clones 122 and 116, respectively).

Percentages of callus formation differed significantly (p=0.0001) among clones in Experiment I (Figure 3.2 and Table 3.4). Clones 122 and 205, intermediate in levels of branch dieback, responded with significantly greater amounts of callus formation (17.7% and 16.7%, respectively) than the other clones (Table 3.5). Because callus formation was associated with only one (puncture wound) of the three wounding techniques, the data were too limited to evaluate in Experiment II. The relative ranking of clones by the amounts of callus formation, however, is similar between both experiments (Figures 3.2 and 3.3).

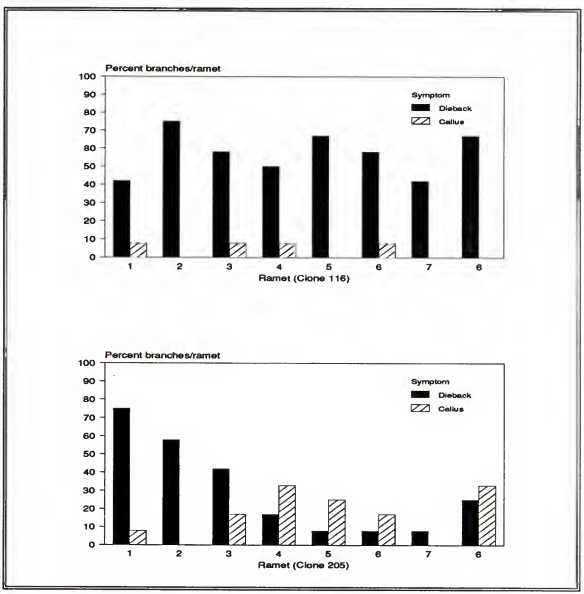

Figure 3.4. Branch dieback and callus formation among ramets of four slash pine clones inoculated with the pitch canker fungus in Experiment I.

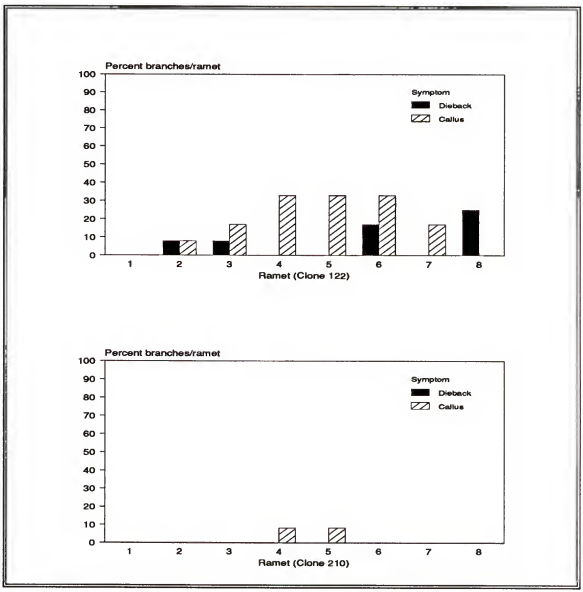

Figure 3.4--continued.

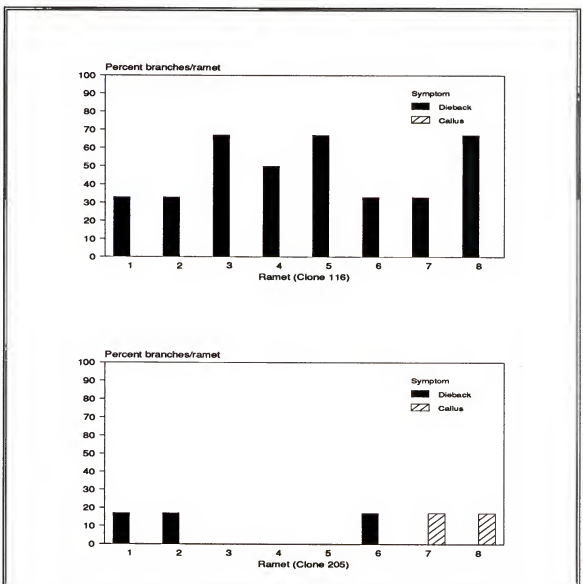

Figure 3.5. Branch dieback and callus formation among ramets of four slash pine clones inoculated with the pitch canker fungus in Experiment II.

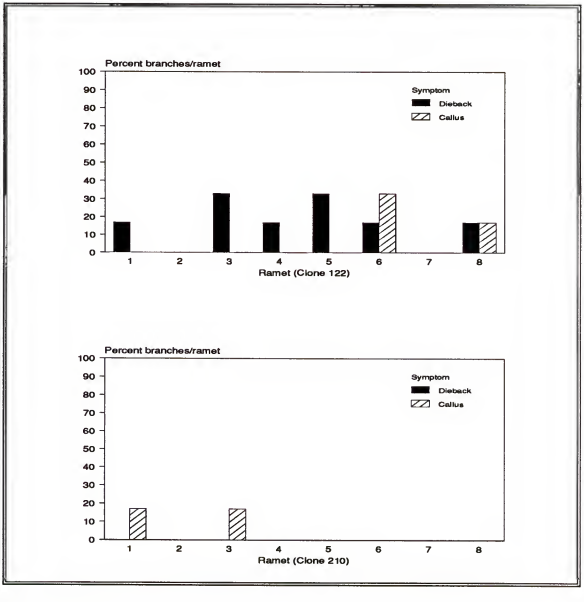

Figure 3.5--continued.

Table 3.4. Effects of slash pine clone, inoculum source, and inoculum concentration on callus formation following inoculation with the pitch canker fungus.

Analysis of variance				
Source	df	SS	F value	Pr. > F
Clone	3	1.92	7.66	0.0001
Inoculum source	3	0.34	1.36	0.2254
Inoculum concen.	2	0.30	1.77	0.1714
Clone*Source	9	0.90	1.19	0.2987
Clone*Concen.	6	0.54	1.07	0.3813
Concen.*Source	6	0.75	1.48	0.1831
Clone*Src.*Conc.	18	2.17	1.44	0.1097
Error	336	28.13		

Variation in the percentages of callus formation was relatively consistent among ramets within clones ranging from 0.0% to 8.3% of the branches in clones 210 and 116 and 0.0% to 33.3% in both 122 and 205 in Experiment I (Figure 3.4). The infrequent occurrence of callus formation in the second experiment precluded similar observations (Figure 3.5).

Only clones 116 and 122 responded with an adequate number of cankers for analysis of colonized host tissue. These clones differed marginally in the amount of total canker length in both Experiments I and II ($p=0.0526$ and $p=0.0570$, respectively) (Tables 3.6 and 3.7).

Inoculum source. Overall, levels of branch dieback did not differ significantly among pitch canker inoculum sources (Table 3.2). Inoculum sources G and

Table 3.5. Callus formation among slash pine clones inoculated with the pitch canker fungus in Experiment I.

Clone	Callus formation[a]
	Percent
122	17.7 a
205	16.7 a
116	4.2 b
210	2.1 b

[a]Means with the same letter are not significantly different (p=0.05).

NV caused the greatest amount of branch dieback (37.5% and 34.7%, respectively) and source OV was the lowest with 25.0% (Figure 3.6).

Inoculum sources significantly interacted with host clone (p=0.0001) with respect to the amount of branch dieback (Table 3.2). A different inoculum source was associated with the highest level of branch dieback for each responding clone; however, inoculum source effects were significant only for clones 122 and 205 (Figure 3.7 and Table 3.8). Inoculation of clone 122 with source NV resulted in more than twice the amount of branch dieback than the other sources but was statistically similar to source OV. For clone 205, the percentage of branch dieback was significantly greater only for inoculum source G.

The amount of callus formation did not differ significantly among inoculum sources (Table 3.4). The greatest difference in the percentage of callus formation

Table 3.6. Effects of slash pine clone, inoculum source, and inoculum concentration on colonization of host tissue following inoculation with the pitch canker fungus in Experiment I.

Source	Significance probabilities[a] Canker length[b]	Colonized tissue[c]
Clone[d]	0.0526	0.1043
Inoculum source	0.4097	0.4003
Inoculum concentration	0.7186	0.9273
Clone*Source	0.5272	0.4560
Clone*Concen.	0.7830	0.9369
Source*Concen.	0.1083	0.3515

[a]Prob. > F.

[b]Total canker length measured proximal from the point of inoculation.

[c]Proportion of colonized tissue from inoculation site to proximal node.

[d]Clones 205 and 210 excluded from analysis because of a lack of canker response.

was between source B (18.0%) and source OV (8.3%) (Figure 3.6). Inoculum source influences were not significant when evaluated as total canker length or proportion of colonized tissue (Table 3.6)

Inoculum concentration. Overall, the percentage of branch dieback differed significantly among levels of inoculum concentration (p=0.0001) (Table 3.2). Inoculum concentration also significantly interacted with clone (p=0.0001). Amounts of branch dieback increased as inoculum concentration increased (14.6%,

Table 3.7. Effects of slash pine clone, wounding technique, and season of inoculation on colonization of host tissue following inoculation with the pitch canker fungus in Experiment II.

Source	Significance probabilities[a] Canker length[b]	Colonized tissue[c]
Clone[d]	0.0570	0.0696
Wounding Technique	0.6665	0.6984
Inoculation Season	0.4034	0.7466
Clone*Technique	0.4304	0.6581
Clone*Season	0.7711	0.6993
Technique*Season	0.6153	0.8266

[a]Prob. > F.

[b]Total canker length measured proximal from the point of inoculation.

[c]Proportion of colonized tissue from inoculation site to proximal node.

[d]Clones 205 and 210 excluded from analysis because of a lack of canker response.

35.4%, and 45.8% at 1 X 10^2, 1 X 10^4, and 1 X 10^6 conidia/ml, respectively) but these differences were significant only for those inoculations made at the lowest (1 X 10^4 conidia/ml) concentration (Figure 3.8 and Table 3.9).

The significant clone x inoculum concentration interaction was attributed to increasing differences in the levels of branch dieback to inoculum concentration as the relative response level for each clone increased (Figure 3.9). When analyzed separately by clone, differences in the amounts of branch dieback relative to

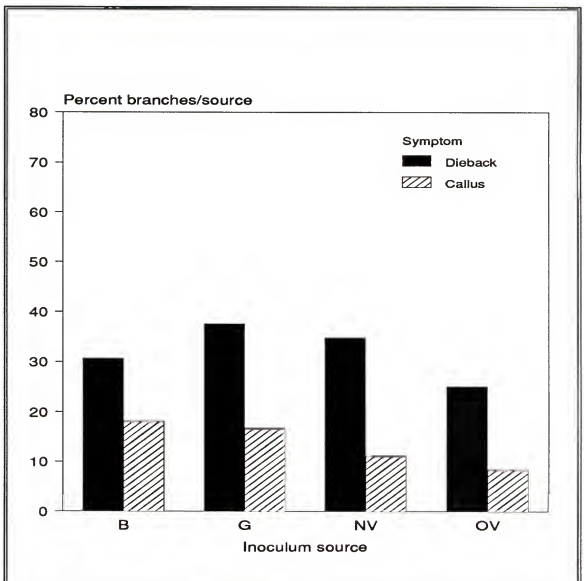

Figure 3.6. Branch dieback and callus formation of slash pines inoculated with four sources of the pitch canker fungus. Data for nonresponding clone 210 excluded.

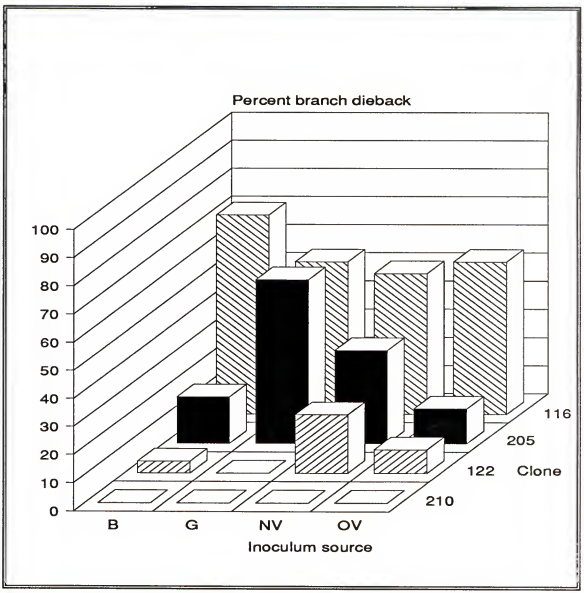

Figure 3.7 Branch dieback of four slash pine clones inoculated with four sources of the pitch canker fungus.

Table 3.8. Branch dieback among sources of the pitch canker fungus inoculated into slash pine clones.

Inoculum source[a]	Total[b]	Clone 116	Clone 122	Clone 205	Clone 210
		Percent branch dieback[c]			
G	37.5	54.2	0.0 b	58.3 a	0.0
NV	34.7	50.0	20.8 a	33.3 b	0.0
B	30.6	70.8	4.2 b	16.7 b	0.0
OV	25.0	54.2	8.3 ab	12.5 b	0.0
Prob. > F	0.2252	0.3236	0.0640	0.0015	ns

[a]B = Franklin, 1982; G = Gilchrist, 1982; NV = Volusia, 1982; OV = Volusia, 1977.

[b]Clone 210 removed from determination of total.

[c]Means with the same letter are not significantly different (p=0.05).

inoculum concentration were significant (p=0.0001) only for the most responsive clone (116) (Table 3.9). For this clone, differences in the amount of branch dieback were significant among all levels of inoculum concentration. Clone 205 responded similar to clone 116 although differences in the percentage of branch dieback were not significant. Branch dieback response in clones 210 and 122 appeared unaffected by inoculum concentration.

The percentage of callus formation did not vary significantly among levels of inoculum concentration although this response increased slightly with increasing levels of inoculum concentration (Figure 3.8 and Table 3.4). Inoculum

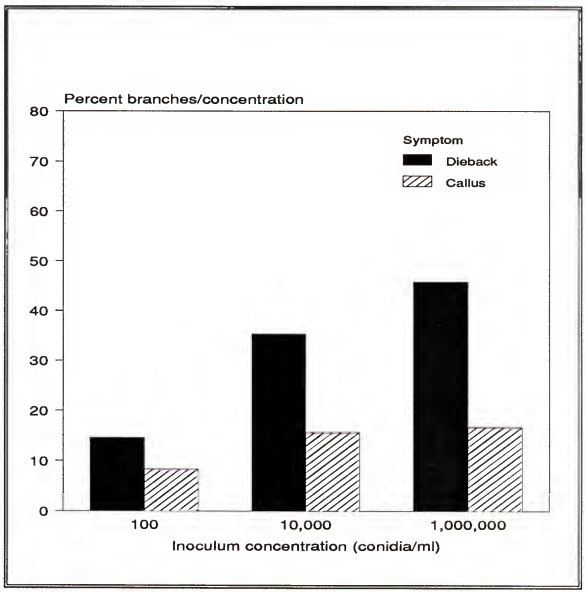

Figure 3.8. Branch dieback and callus formation of slash pines inoculated with three inoculum concentrations of the pitch canker fungus. Data for nonresponding clone 210 excluded.

Table 3.9. Branch dieback among inoculum concentrations of the pitch canker fungus inoculated into slash pine clones.

Inoculum concentration[a]	Total[b]	Clone			
		116	122	205	210
		Percent branch dieback[c]			
1 X 10^6	45.8 a	87.5 a	9.4	40.6	0.0
1 X 10^4	35.4 a	65.6 b	12.5	28.1	0.0
1 X 10^2	14.6 b	18.8 c	3.1	21.9	0.0
Prob. > F	0.0001	0.0001	0.3938	0.2145	ns

[a]Conidia/ml aqueous suspension.

[b]Clone 210 removed from determination of total.

[c]Means with the same letter are not significantly different (p=0.05).

concentration influences were not significant evaluated as total canker length or proportion of colonized tissue (Table 3.6).

Wounding technique. The percentage of branch dieback varied significantly among the three wounding techniques (p=0.0001) (Table 3.3). Inoculations using hypodermic needle puncture wounds resulted in significantly greater amounts of branch dieback (43.8%) than wounding by fascicle removal wounding (22.9%), both of which were significantly greater than nonwounded inoculations (4.2%) (Figure 3.10 and Table 3.10).

The significant host clone x wounding technique interaction (p=0.0007) was the result of the difference in magnitude of branch dieback among clones to the

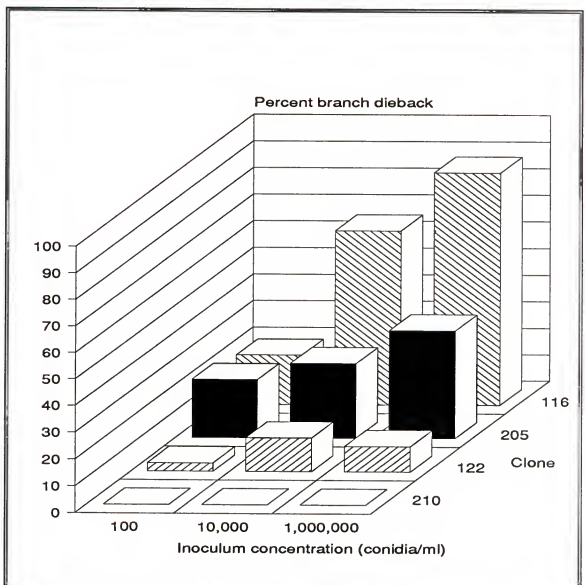

Figure 3.9. Branch dieback of four slash pine clones inoculated with three inoculum concentrations of the pitch canker fungus.

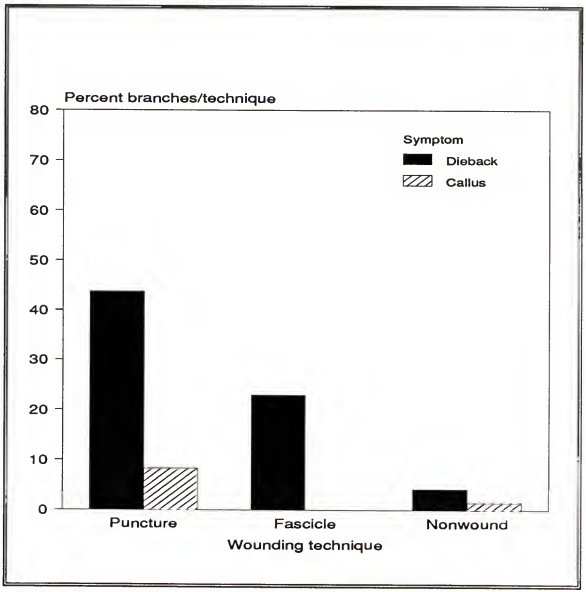

Figure 3.10. Branch dieback and callus formation of slash pines inoculated with aqueous suspensions of the pitch canker fungus using three wounding techniques. Data for nonresponding clone 210 excluded.

Table 3.10. Branch dieback among slash pine clones and wounding techniques used to inoculate the pitch canker fungus.

Wounding technique	Total[a]	Clone			
		116	122	205	210
		Percent branch dieback[b]			
Puncture	43.8 a	81.3 a	31.3 a	18.8 a	0.0
Fascicle remove	22.9 b	50.0 b	18.8 ab	0.0 b	0.0
Nonwounded	4.2 c	12.5 c	0.0 b	0.0 b	0.0
Prob. > F	0.0001	0.0002	0.0524	0.0217	ns

[a]Clone 210 removed from determination of total.

[b]Means with the same letter are not significantly different (p=0.05).

different inoculation techniques (Table 3.3). When analyzed separately, the effects of wounding technique were significant for each responding clone (Figure 3.11 and Table 3.10). In these clones, the amount of branch dieback was greatest from puncture wound inoculations, intermediate with fascicle removal wounding, and lowest with nonwounded inoculations.

Data were insufficient to evaluate the effects of wounding technique on the quantity of callus formation. Only inoculations made with puncture wounds resulted in callus formation (Figure 3.10). None of the branches wounded by fascicle removal developed callus. Only one branch inoculated without intentional wounding exhibited callus formation but this infection was associated with a naturally occurring infection that apparently developed at the base of the shoot.

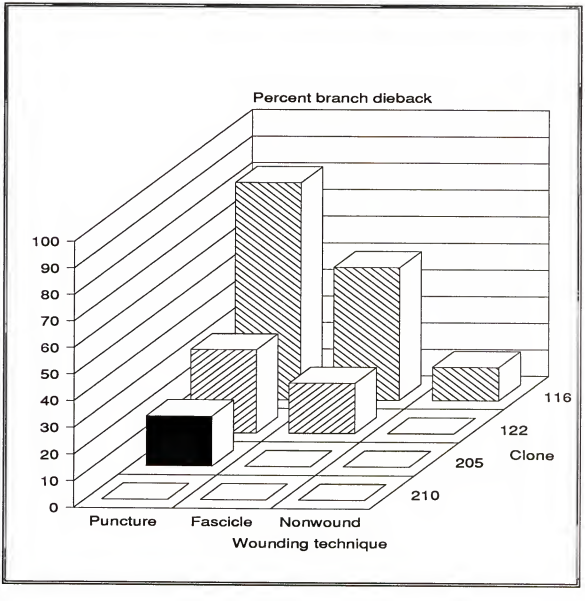

Figure 3.11. Branch dieback of four slash pine clones inoculated with aqueous suspensions of the pitch canker fungus using three wounding techniques.

Total canker length or the proportion of colonized tissue did not significantly vary among inoculation techniques (Table 3.7).

Season of inoculation. Season of inoculation did not significantly influence levels of branch dieback (Table 3.3). The amount of branch dieback was the same (23.6%) for summer and fall inoculations (Figure 3.12).

The significant wounding technique x season of inoculation interaction ($p=0.0344$) relates to changes in the greatest amount of branch dieback between seasons of inoculation for each wounding technique (Table 3.3). Branch dieback was greater following summer inoculations with the puncture wound technique, and greater for fall inoculations with the fascicle removal wound and nonwounded techniques; however, these differences were not significant (Figure 3.13 and Table 3.11).

Amounts of callus formation were minimal for both summer and fall seasons of inoculation (5.6% and 1.6%, respectively) (Figure 3.12). The main effects of season of inoculation and all related interactions were not significant evaluated as total canker length and as a proportion of colonized tissue (Table 3.7).

Pathogen recovery from inoculated test branches. Pathogen recovery increased with increasing severity of symptom expression (Table 3.12). The pitch canker fungus was present in 30% or more of asymptomatic inoculated branches. In this symptom type, positive isolation for clone 205 was nearly twice (62.5%) that of any other clone. In all cases (100%), recovered Fusarium subglutinans isolates were pathogenic on pine.

Inoculum quality. When tested subsequent to field inoculations, spore germination was always equal to or greater than 80% (Table 3.13). Pathogenicity was verified in every instance.

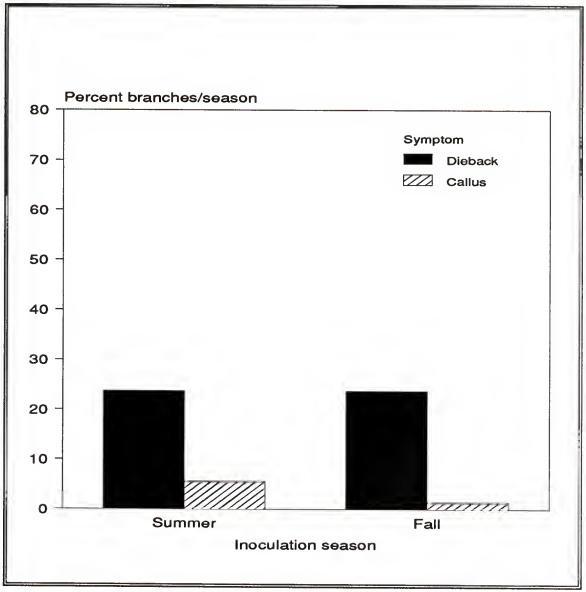

Figure 3.12. Branch dieback and callus formation of slash pines inoculated with the pitch canker fungus in two seasons. Data for nonresponding clone 210 excluded.

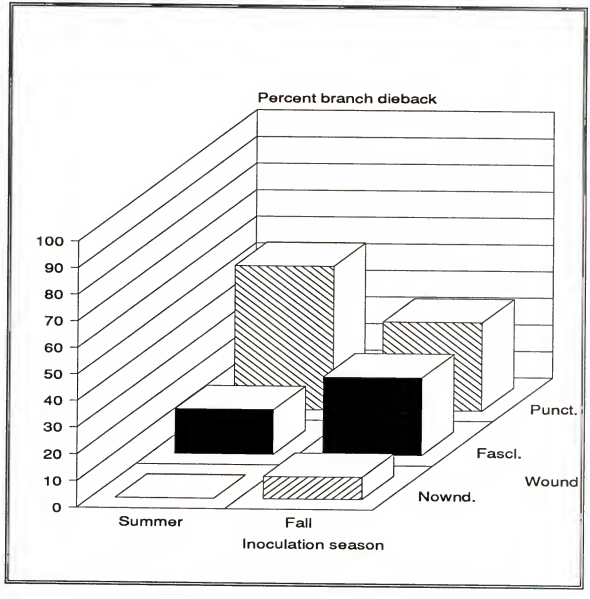

Figure 3.13 Branch dieback of slash pines inoculated with the pitch canker fungus at two seasons using three wounding techniques.

Table 3.11. Branch dieback of slash pines inoculated with the pitch canker fungus among wounding techniques and seasons of inoculation.

Season of inoculation[a]	Total[b]	Wounding technique		
		Puncture	Fascicle removal	Non-wounded
		Percent branch dieback		
Summer	23.6	54.2	16.7	0.0
Fall	23.6	33.3	29.2	8.3
Prob. > F	1.0000	0.0870	0.2535	0.1341

[a]Summer inoculations - 22 Sep. to 7 Oct. 1982, Fall inoculations - 12-13 Nov. 1982.

[b]Clone 210 removed from determination of total.

Control inoculations. Symptoms (including callus formation) were not apparent on all but one of the nonwounded, noninoculated control branches. In clone 205, a diffuse canker from an active infection was observed in association with a deodar weevil feeding pit on one of the control branches. The pitch canker pathogen was isolated from this branch, and its pathogenicity was verified.

Table 3.12. Isolation of Fusarium subglutinans from inoculated slash pine branches and pine pathogenicity verification.

Symptom type	Host clone			
	116	122	205	210
Asymptomatic				
N	14	26	16	31
Isolation[a]	35.7	38.5	62.5	32.3
Path. ver.[b]	100.0	100.0	100.0	100.0
Callus				
N	1	4	2	0
Isolation	100.0	25.0	50.0	--
Path. ver.	100.0	100.0	100.0	--
Cankered/ Alive				
N	2	9	11	1
Isolation	100.0	88.9	72.7	100.0
Path. ver.	100.0	100.0	100.0	100.0
Dieback				
N	75	15	27	0
Isolation	93.3	100.0	100.0	--
Path. ver.	100.0	100.0	100.0	--

[a]Recovered isolates expressed as a percent of total (N).

[b]Pathogenicity verifications expressed as a percent of positive isolation.

Table 3.13. Pitch canker inoculum germination and pathogenicity verification.

Inoculum[b] source	Inoculum germination and pathogenicity verification[a] Field inoculation date						
	9/20	9/22	9/24	10/1	10/7	11/8	11/9
	Percent spore germination						
B	100/P	100/P	95/P	98/P	87/P		
G	100/P	100/P	90/P	96/P	87/P		
NV	100/P	100/P	90/P	100/P	90/P		
OV	100/P	100/P	95/P	94/P	80/P		
S[c]	98/P	100/P	95/P	100/P	94/P	95/P	96/P

[a]P = pathogenicity verified.

[b]Inoculum concentration tested at 1 X 10^6 conidia/ml in Experiment I.

[c]Pathogen source NV at 1 X 10^5 conidia/ml in Experiment II.

Discussion

Host Genotype

Slash pine clones varied significantly in pitch canker disease expression when evaluated by artificial inoculation and disease surveys of naturally occurring infections in a seed orchard. Artificial inoculations resulted in relatively consistent levels of branch dieback among ramets for most host clones indicating this response is an expression of genetic susceptibility. The pitch canker fungus was

consistently recovered from asymptomatic inoculated shoots in each clone 8 mo after inoculation demonstrating the observed differences were not the result of disease escape. The large difference in the amount of branch dieback response to artificial inoculation, from the near-immune response of clone 210 (0%) to over 57% for clone 116, suggests a potentially large selection differential for pitch canker resistance. Rockwood et al. (1988) also reported differences in pitch canker resistance can be identified using artificial inoculation.

The similar host response among the two survey techniques and corresponding inoculation experiments suggests evaluating host genotypes by observed amounts of naturally occurring branch dieback may provide reliable estimates of relative pitch canker susceptibility. This relationship is observed for a limited number of clones in a seed orchard environment, however, and may change when different clones are evaluated or for half-sib progeny growing under plantation environments. In loblolly pine, Kuhlman et al. (1982) reported clones rated as susceptible to pitch canker during a previous seed orchard survey were also more susceptible following artificial inoculation than those rated as either intermediate or resistant.

Rockwood et al. (1988) estimated potential genetic gains of at least 11.0% for increasing pitch canker resistance of progeny from seed production areas developed by roguing heavily infected stands. If branch dieback is environmentally insensitive then collection of seed by clone or the rouging of seed orchard clones using disease survey techniques or artificial inoculation procedures could also provide substantial genetic gains.

No apparent relationship in the amount of branch dieback response existed between geographic sources of the inoculum and the host. Inoculum source G from north central Florida interacted significantly with host clone 205 from the

same region but did not produce any branch dieback in clone 210, also from the same region. These results suggest a substantial tree to tree variation in pitch canker susceptibility exists for slash pine, also speculated by Bethune and Hepting (1963), in addition to the reported geographic sources of variation (Blakeslee and Rockwood 1978, Rockwood et al. 1988).

The amount of callus formation in response to inoculation also varied significantly among slash pine clones. While this trait does not appear to be directly related to branch dieback it may be an expression of a mechanism of resistance. Visible callus formation was absent in control branches inoculated with sterile water.

Differences in pitch canker resistance in Virginia pine were suggested to be related to the timing and quantity of callus tissue produced (Barrows-Broaddus and Dwinell 1984). The significantly lower percentages of callus produced in both the most susceptible (116) and resistant (210) slash pine clones may be related to rate of host response to pathogen invasion, slow in the susceptible clone allowing extensive colonization of the host, and fast in the resistant one, resulting in limited development of associated callus. The significantly greater callus formation in clones with intermediate levels of branch dieback also suggests callus formation may be related to an intermediate host response rate between resistant (asymptomatic) and susceptible (branch dieback).

Slash pine clones differed only marginally in canker length (measured from the point of inoculation to the proximal canker margin). Blakeslee et al. (1979) reported, however, that the same measure of canker length was consistent with pitch canker susceptibility expressed as percentage of shoot dieback in slash pine seedlings. In both of the inoculation experiments, invasion by the pathogen was often observed to be arrested at the nodes in some clones while in others,

colonization continued through the node (especially clone 116). If evaluated prior to the time when pathogen invasion had reached the node, canker length may be a meaningful expression of host response.

Inoculum Source

Significant variation in levels of branch dieback was observed for specific host clone-inoculum source combinations. The most susceptible and most resistant slash pine clones appeared insensitive to differences in inoculum source. The strong interaction between host and pathogen shown in this study suggests inoculum source should be considered when evaluating slash pine susceptibility to pitch canker. Previous reports generally indicate minimal differences in virulence among diverse pitch canker pathogen sources when evaluated genetically undefined host material (Barrows-Broaddus and Dwinell 1979, 1983, 1984, Dwinell and Barrows-Broaddus 1983). Barrows-Broaddus and Dwinell (1985) reported virulence differences only among individual Fusarium subglutinans isolates from loblolly pine when inoculated into Virginia pine.

The lack of association between higher frequencies of branch dieback and inoculum sources from stands of high disease intensity (e.g., OV and B) suggests that pitch canker disease outbreaks may be related more to environmental influences or genetic susceptibility of planted slash pine genotypes.

Inoculum Concentration

Aqueous inoculum suspensions were effective in causing branch dieback. Higher inoculum concentrations yielded higher levels of branch dieback but the

similar branch dieback response between inoculations with concentrations of 1 X 10^4 and 1 X 10^6 conidia/ml indicate that 1 X 10^4 is the minimum concentration required for consistent disease expression.

Wounding Technique

Although infection occurred (4.2%) in the absence of deliberate wounding, the large and significant difference with respect to the puncture wounding technique (43.8%) confirms the wound-parasitic nature of Fusarium subglutinans (Hepting and Roth 1946). Infections on shoots receiving inoculum without intentional wounding (nonwounded treatment) may have arisen from accidental or naturally occurring wounds.

Clone 210 did not develop branch dieback symptoms even when wounded by two different techniques. These results strengthen the observation that at least one component of pitch canker disease resistance is related to defense mechanisms regulating pathogen invasion of host tissue and not to the predisposition of the host to wounding (i.e., bark thickness, etc.).

Puncture wound inoculations provided higher levels of host response indicating consistent exposure of the pathogen to the host and therefore may be most appropriate for evaluation of pitch canker resistance. For example, if only the fascicle removal wound technique was used, clone 205 would have been evaluated as near-immune (equal to clone 210) instead of intermediate in pitch canker resistance as estimated from the two seed orchard surveys and from puncture wound inoculations.

The absence of external callus formation with inoculations using fascicle wounding suggests that the development of callus is related to the type of wound

which the fungus enters. Relatively larger puncture wounds may allow more inoculum into the wound thus altering host response to infection (triggering host response differently, influencing the capacity of the tree to respond, affecting invasion rate, etc.). The smaller fascicle wounds may allow less inoculum to enter susceptible tissues allowing the tree to control infection more easily.

Season of Inoculation

The lack of overall differences in the amounts of branch dieback between summer and fall inoculations suggests that tissue susceptibility is maintained despite changes in environment and host phenology. These findings are in contrast to seasonal effects in loblolly pine where Kuhlman et. al. (1982) reported increased branch dieback from fall and winter inoculations. Seasonal susceptibility differences may have been detected in this study, however, with more inoculations and a greater time span between seasons of inoculation.

The pitch canker fungus was consistently recovered from branches with callus formation. The fate of viable inoculum within the callus formation response is unknown. By appearance, the tree contained the fungus and progressive invasion was arrested. It may be possible that seasonal changes in host susceptibility not apparent in this study may allow colonization to continue eventually leading to branch death. These circumstances seem improbable, however, because callus formation symptoms have not been observed on shoots killed by naturally occurring pitch canker infections.

Summary

Techniques for reliable artificial inoculation of mature slash pine with the pitch canker fungus were identified using aqueous conidial suspensions of Fusarium subglutinans dispensed into needle puncture wounds on branches previously surface sterilized by misting with 95% ethanol. Although successful infections occurred at inoculum concentrations of 1×10^2 conidia/ml, minimum concentrations of 1×10^4 conidia/ml were required to assure against disease escape. Disease expression did not differ between summer and fall inoculation trials.

Expression of branch dieback appears to be under strong genetic control. Among slash pine clones, branch dieback response to artificial inoculation ranged from nearly immune (0%) to very susceptible (57.3%) and differences among sources of the pitch canker fungus were important only in certain specific host-pathogen combinations.

Estimates of pitch canker susceptibility from two survey techniques of naturally occurring infections in a slash pine seed orchard closely agreed with results from artificial inoculation trials. Furthermore, the pitch canker fungus was consistently recovered from both symptomatic and asymptomatic branches 8 mo after inoculation demonstrating disease escape was not responsible for the observed differences. The basis for pitch canker resistance in slash pine appears to involve containment of pathogen invasion.

CHAPTER IV

INFLUENCES OF PATHOTYPE AND SITE FERTILIZATION ON SYMPTOM EXPRESSION OF FIELD-GROWN, HALF-SIB SLASH PINE PROGENY INOCULATED WITH THE PITCH CANKER FUNGUS

Introduction

In slash pine, pitch canker disease expression is mediated by environmental factors and genetic relationships of host-pathogen interactions. Several of these response-regulating factors have been studied in the past, however, interactions among these variables, important in developing comprehensive disease management strategies, are unknown.

Differences in virulence of pine pathogenic strains of Fusarium subglutinans may be important but evidence is not conclusive. Several reports indicate differences in virulence among various pathogen isolates evaluated as the amount of shoot dieback were not significant (Barrows-Broaddus and Dwinell 1979, 1983, 1984, Dwinell and Barrows-Broaddus 1983). Statistically significant differences in virulence were reported, however, between two pitch canker isolates inoculated into Virginia pine but not so when inoculated into loblolly pine (Barrows-Broaddus and Dwinell 1985).

Genetic resistance to pitch canker, expressed as the percentage of shoot dieback or canker length, is reported for slash pine (Barrows-Broaddus and Dwinell 1979, Blakeslee and Rockwood 1978, Blakeslee et al. 1979, McRae et al. 1985, Rockwood et al. 1988). Callus formation was also noted in the absence of

dieback from inoculation with the pitch canker fungus for Virginia pine (Barrows-Broaddus and Dwinell 1984), slash pine (G. M. Blakeslee, personal communication), and loblolly pine (Fraedrich 1979), however, the response was not quantitatively evaluated.

Numerous pitch canker outbreaks on susceptible pine species are associated with increases in site fertility. During a major disease outbreak, Phelps and Chellman (1976) reported levels of pitch canker infection and damage in slash pine seed orchards and plantations in Florida were greatest on fertilized sites. Severe disease outbreaks are also reported in seed orchards of other susceptible pine species where intensive culture usually included fertilization (Dwinell et al. 1977, Kelly and Williams 1982, Oak et al. 1983). An increasing gradient of pitch canker incidence in a young slash pine plantation coincided with increasing levels of foliar nutrients and soil fertility attributed to a nearby poultry operation (Cleason and Smith 1978).

Results from field fertilization studies to assess the effects of fertilization on pitch canker disease development are inconclusive. Significant increases in the incidence of pitch canker symptoms from natural infections are reported for slash pine fertilized with nitrogen or nitrogen plus phosphorus (Lowerts et al. 1985, Wilkinson et al. 1977). In addition, disease severity (evaluated as tree mortality) in a previously infected slash pine stand intensified significantly following applications of large amounts of urea plus phosphorus, however, these effects were counteracted when K plus minor elements were also applied (Fisher et al. 1981). Mean canker length of inoculated slash and loblolly pines fertilized with a combination of nitrogen, phosphorus, and potassium were significantly longer than on unfertilized trees (Fraedrich and Witcher 1982). In a companion study, mean canker length on slash and loblolly pines did not significantly differ among

treatments (Fraedrich 1979). Anderson and Blakeslee (1984) also reported no significant increases in natural disease levels of slash pine with applications of nitrogen and phosphorus at recommended rates. In the only study examining application rate, Fisher et al. (1981) found levels greater than 200 lbs/ac N (224.16 kg/ha) and 100 lbs/ac P (112.08 kg/ha) applied to a spodic site significantly increased pitch canker disease severity.

Huber and Watson (1974) report nitrogen either as ammonium or nitrate is generally more influential in affecting disease than nitrogen availability. The influences of nitrogen form has not been evaluated with respect to pitch canker although increases in disease expression has been reported for studies using either ammonium nitrate or urea as the nitrogen source.

Fertilization may interact with pitch canker expression in slash pine by influencing genetic mechanisms of resistance. Fertilization interactions with slash pine genotypes on total accumulation and within plant distribution of nitrogen and phosphorus were reported by Jahromi et al. (1976). Knowledge of recommended forest fertilization influences on pitch canker resistance of slash pine genotypes exposed to different pathotypes of the pitch canker fungus would help identify pest management strategies for intensive pine culture and provide further understanding of the disease biology.

The objectives of this research were to 1) classify phenotypic expressions of genetic pitch canker resistance in slash pine; 2) determine the influences of forest fertilization practices using phosphorus, nitrogen, and forms of nitrogen, on disease expression of slash pine genotypes inoculated with diverse sources of the pitch canker fungus; and 3) evaluate the relationships of foliar elements (N, P, K, Ca, Mg, Al, Cu, Fe, Mn, and Zn), height growth, host genotype, and fungus pathotype to disease expression of slash pines inoculated with the pitch canker fungus.

Methods and Materials

Field Site

The field study was located on a typical south Florida flatwoods site in Volusia County, Florida, chosen because of a history of severe pitch canker outbreaks and the economic importance of slash pine culture on these sites. The soil was a Smyrna fine sand, a sandy, siliceous, hyperthermic, Aeric Haplaquod. The study area was situated within a 405 ha (1000 ac) block where the prior slash pine plantation was harvested prior to economic maturity because of unusually high pitch canker associated mortality.

Emphasis in selection of this site was placed on uniformity of soils, topography, drainage, and vegetation. The study site was bordered on one side at a distance of 91 m (300 ft) by a 20-yr-old slash pine plantation exhibiting little noticeable pitch canker infection. The study site was operationally site-prepared 6 mo prior to planting by chopping, burning, and bedding.

Fertilization Treatments

Influences of phosphorus, nitrogen levels, and forms of nitrogen commonly used in the fertilization of slash pine plantations and seed orchards in the southeastern U.S. were evaluated among six fertilizer treatment combinations.

The fertilizer treatments consisted of 168.12 and 448.32 kg/ha (150 and 400 lbs/ac) N equivalent ammonium sulfate [$(NH_4)_2SO_4$], alone and in combination with 112.08 kg/ha (100 lbs/ac) P equivalent triple super phosphate (TSP); 448.32 kg/ha (400 lbs/ac) N equivalent sodium nitrate ($NaNO_3$) plus 112.08 kg/ha (100 lbs/ac) P

Table 4.1. Designation of treatment code to fertilization application.

Block	Fertilization treatment			
	N	P	N	P
	Kg/ha		Lbs/ac	
T1	0	0	0	0
T2	168.12 NH_4	0	150 NH_4	0
T3	448.32 NH_4	0	400 NH_4	0
T4	168.12 NH_4	112.08	150 NH_4	100
T5	448.32 NH_4	112.08	400 NH_4	100
T6	448.32 NO_3	112.08	400 NO_3	100

equivalent TSP; and an ambient fertility reference (Table 4.1). To compensate for the Ca fraction (13%) in TSP, 72.85 kg/ha (65 lbs/ac) Ca equivalent calcium carbonate ($CaCO_3$) was applied on treatment blocks not receiving TSP. For the same reason, wettable sulfur was applied to treatment blocks to compensate for the sulfur concentration in $(NH_4)_2SO_4$ (491.02 and 306.89 kg/ha [438.10 and 273.81 lbs/ac] S equivalent for those blocks treated without $(NH_4)_2SO_4$, and with 168.12 kg/ha (150 lbs/ac) N of $(NH_4)_2SO_4$, respectively). These treatment combinations were randomly assigned among adjacent 0.125 ha (0.30 ac) blocks.

All fertilizer treatments were applied 2 years postplanting, on 13-15 Mar 1983, prior to initiation of the current season's growth. The nitrogen and sulfur treatments were equally divided into two applications; the second application occurring on 9 May 1983 just prior to the second growth flush of the season. A hand cyclone spreader was used to uniformly distribute the fertilizer. Areas between tree rows were mowed at 2 wk intervals to minimize possible confounding effects of competing vegetation.

Host Genotypes

Host genotype effects were examined among 12 half-sib slash pine progenies selected to represent a wide range of phenotypic pitch canker expression (Appendix). Three progeny replicates, consisting of all 12 slash pine progenies, were planted within each fertilizer treatment block. Replicates consisted of randomly located five-tree row plots of each progeny. A row of border trees separated replicates on the sides and ends; fertilizer treatment blocks were equally isolated. The entire study was planted with 1-0 nursery stock on a 2.44 X 3.04 m (8 X 10 ft) spacing in January 1981.

Field Inoculations

Pathogen variability of Fusarium subglutinans was evaluated among three pathotypes. The pathotypes originated from geographic regions within Florida characterized by different levels in stand disease expression (one isolate was chosen from the five used for each of the three pathogen populations in Chapter 3). These pathotypes, cultured from infected slash pine tissue, originated from 1) a 15-yr-old plantation in 1982 from Gilchrist County, Florida, characterized by occasional tree infections with light crown damage (P1), 2) a stock culture isolate from Volusia County, Florida, collected during a major disease outbreak in 1977 (P2), and 3) a 10-yr-old plantation in 1982 from the same area in Volusia County, but with scattered infections of light severity (P3).

Active cankers from the various regions were cultured on PCNB media (Nash and Snyder 1962) at 24 C and a 12 hr photoperiod for 10-14 days. Suspected Fusarium subglutinans colonies were transferred to carnation water agar

media (CWA) for morphological identification under the same cultural conditions. Fusarium subglutinans isolates were single-spored, identified, and their pathogenicity verified by inoculating shoots of 18-mo-old slash pine seedlings by placing a droplet (ca. 0.03 ml) of 1 X 10^6 conidia/ml aqueous suspension into a needle puncture wound. Isolates were identified as pine pathogenic upon isolation of Fusarium subglutinans from dead tissues proximal to the inoculation site.

Since isolated in 1977 until used in 1982, the stock culture isolate was maintained on potato dextrose agar media in culture tubes at 1 C in the dark. To rejuvenate virulence that may have been reduced during storage, the stock isolate was cultured on CWA and inoculated into 1-yr-old slash pine seedlings following the same procedures for pathogenicity verification. Isolations were made from the proximal extension of lethal cankers. Recovered isolates were again single-spored, identified, and pathogenicity verified for use as a pathotype.

The three pathotypes were grown on CWA for 10-12 days at 24 C and a 12 hr photoperiod. Conidia from these cultures were added to sterile deionized water and concentrations were adjusted to 1 X 10^6 conidia/ml using a hemocytometer. Approximately equal proportions of macro- and microconidia were obtained by selectively scraping sporodochia and aerial portions of the colonies bearing microconidia. Fresh inoculum was prepared each field inoculation day from cultures of equal age.

Prior to inoculation, branch surfaces were misted to runoff with 95% ethanol and air dried. Puncture wounds were created with a hypodermic needle on opposing sides of the test branch midway along the flush and a droplet (ca. 0.03 ml) of inoculum was dispensed into each wound (inoculation procedures, Chapter 3).

Each host tree was challenged with all three isolate sources; each source was inoculated into a separate branch. Within each tree, test branches of uniform physical and phenological condition, typically the third flush of the season, ca. 1.5 cm in diameter, were chosen among dominant laterals in the upper crown.

Trees were inoculated during a 16 day period (12-27 Sep 1983). One randomly selected progeny replicate within a fertilizer block was inoculated each day. Germinability of inoculum was evaluated subsequent to each field inoculation day by placing droplets of inoculum suspension on 2% water agar and incubating in the dark for 12 hrs.

Experimental Design

Randomly selected fertilization treatments were applied over three adjacent host progeny replicates to reduce potential contamination among fertilizer treatments through roots extending into adjacent treatment blocks. Fertilization treatments were considered as whole plots and the host progeny replicates were subsamples or pseudoreplicates (Hurlbert 1984). Lack of spatial interspersion of fertilization treatments prohibited inferential statistics to test for pure fertilization effects. Therefore, fertilization treatment/location effects are referred to as fertilization effects in the text. Host progenies were evaluated as subplots within each fertilization treatment location; pathogen sources, each inoculated into the same tree, were evaluated as sub-subplots.

Location influences among and within fertilization treatments were minimized by selecting a uniform field site on a number of criteria. Prefertilization tree heights and foliar composition were evaluated to detect site influences on tree response. In this preliminary site analysis, true replication existed among

fertilization treatment locations. Fertilizer materials were uniformly applied within fertilization treatment locations.

Foliar Analysis

Foliage samples were collected on 27 Nov 1982, and 30 Sep 1983, to evaluate host nutrition prior to and subsequent to fertilization. Twelve composite host progeny samples were collected for each fertilization block by combining an equal amount of foliage removed from the terminal portion of dominant lateral branches in the upper crown of all trees of each progeny (CRIFF 1985). The samples were oven-dried for 48 hrs at 60-70 C, ground to pass a 2 mm screen mesh, and analyzed for percent N, P, and K, and concentrations of Ca, Mg, Al, Cu, Fe, Mn, and Zn by the Forest Soils laboratory of the Soil Science Department, University of Florida, Gainesville, Florida.

Tree Height Measurement

Tree heights were measured at the end of the growing season prior to fertilization (ca. 2-yr-old), and at the time of inoculation near the end of the next growing season, 6 mo postfertilization.

Weather Monitoring

Precipitation was monitored with a recording (weight) rainfall gauge installed in the center of the study plot. Rainfall data was collected starting 1 mo prior to fertilization and continued until the study was terminated 15 mo later.

Temperature and humidity for the same period was obtained from data collected at the Sanford regional airport, Sanford, Florida, 32 km (20 mi) SE of the research area.

Evaluation of Host Response

Inoculated test branches were harvested on 7-24 May 1984, 8 mo after inoculation, and brought into the laboratory for examination. Progeny replicates were sequentially harvested following the order of inoculation. Tissue response type for both inoculations on each test branch was rated according to the three categories of external symptom expression. The immune-like response type was characterized by lack of external tissue response other than what occurs following wounding, but from which the fungus was consistently recovered. In the callus formation response, a margin of dark-colored hypertrophic callus tissue surrounded the inoculation site appearing to delineate the extent of pathogen invasion. Branch dieback response was identified by a lethal girdling canker resulting from unrestricted host colonization by the fungus.

Pathogen Isolation and Pathogenicity Verification

Isolations were made from 2898 inoculated and 209 control branches. Branch tissue samples were taken from above (distal), within, and below (proximal) the inoculation site, and at the canker margin when appropriate. Tissue samples were surfaced sterilized by dipping into 95% ethanol and flaming before culturing on PCNB media for 14 days at 24 C and a 12 hr photoperiod.

Suspected Fusarium subglutinans colonies were transferred to CWA for positive morphological identification.

Groups of Fusarium subglutinans isolates from immune-like and diffuse canker stem tissue response types were selected for verification of pine pathogenicity. Isolates chosen for testing each of these host response groups consisted of cultures from each host progeny-fungus pathotype combination.

Statistical Analysis

Fertilization treatment locations were evaluated using analysis of variance procedures on prefertilization tree height and foliar N and P concentration (Cramer and Walker 1982, Snedecor and Cochran 1967). Tree height and foliar element concentration subsequent to fertilization were evaluated with an analysis of covariance and the adjusted means compared using the least squares procedure. Both pre- and postfertilization data on tree height and foliar composition data were examined for normality subsequent to analysis. Differences among tissue response types within each double-inoculated branch were evaluated using a paired t-test on response type differences.

Treatment effects of host progeny, fungus pathotype, fertilization treatment, and their interactions were evaluated by an analysis of variance on the angular transformation of the proportion of branch dieback. Treatments with significant interactions were evaluated at each level of the interacting effect. Fertilization treatment, host progeny, and fungus pathotype were evaluated as fixed effects and host progeny replicate with fertilization treatment was evaluated as a random effect. Differences among treatment means were compared with the least significant difference procedure (Cramer and Walker 1982).

Influences of fertilization treatment, fungus pathotype, host progeny, and the relationship of tree height growth, on branch dieback were also evaluated with logistic regression procedures (McCullagh and Nelder 1983). The class variable of fertilization treatment was analyzed along with postfertilization foliar percent of N, P, and K, and concentrations of Ca, Mg, Al, Cu, Fe, Mn, and Zn, which are measures of treatment effects evaluated directly on the sample unit of the inoculated branch. Influences of individual fertilization treatments, fungus pathotypes, and host progenies were evaluated using dummy variables. Stepwise selection was used to identify a significant regression model from these variables. Host progenies were grouped according to significant differences in branch dieback identified with a least-squares procedure from a general linear regression of the selected logistic model. Dummy variables representing the significant progeny and pathotype groups were analyzed using the same logistic regression procedures. The analyses were performed using ANOVA, TTEST, LOGIST, and GLM implementations in SAS (SAS 1983, 1985).

Results

Edaphic and Environmental Variability

Climatic. Monthly averages of temperature and rainfall during the field study period were compared with the previous 30 yrs (Figures 4.1 and 4.2). Average monthly temperatures appeared normal, although precipitation amounts differed somewhat. Rainfall for September 1983 was about half of the 30 yr average; October rainfall was nearly double. Rainfall in subsequent months was

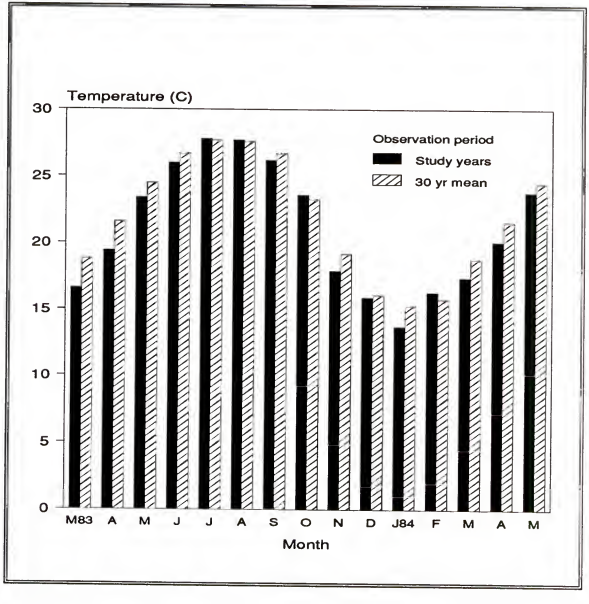

Figure 4.1. Average monthly temperatures for 1983-84 and from the past 30 years.

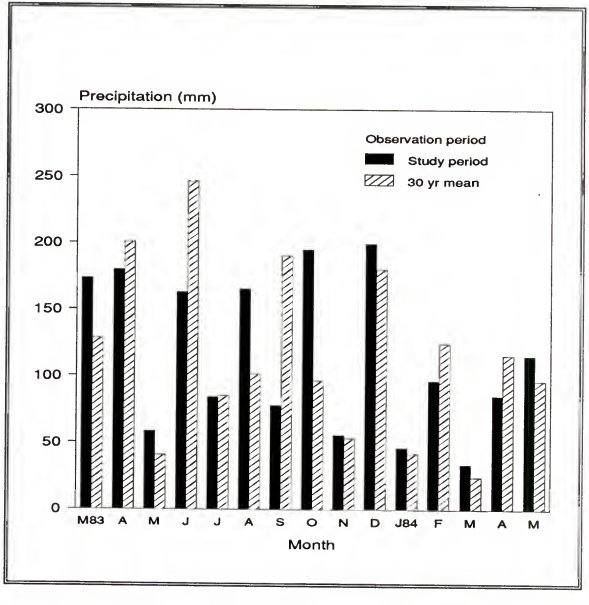

Figure 4.2. Average monthly precipitation for 1983-84 and from the past 30 years.

comparable. Total precipitation from the month of inoculation (September 1983) until the end of the study (March 1984) appeared normal.

Prefertilization tree height. Prefertilization tree heights averaged 1.65 m (range 1.56 to 1.71 m) (Table 4.2). Tree heights did not differ among fertilization treatment locations although progeny and progeny x fertilizer treatment location influences were significant (p=0.0001 and p=0.0171, respectively). A small coefficient of determination (r^2 = 0.22) indicated much of the variation in tree height was unaccounted for in the analysis.

Prefertilization foliar N and P concentration. Prefertilization differences in foliar N and P concentration levels in all treatment locations, while statistically significant (p=0.0003 and p=0.0001 for N and P, respectively), were quite small in actual magnitude and equal to or less than 0.88% and 0.07%, respectively; both levels are considered limiting for slash pine growth (CRIFF 1985) (Tables 4.3 and 4.4). Similar to tree height, a large part of the variation in prefertilization foliar element concentration was unrelated to treatment location influences (r^2=0.51 and r^2=0.47 for N and P, respectively).

Tree height growth response to fertilization. Mean tree height (adjusted for prefertilization variation) differed significantly among fertilization treatments and host progenies (p=0.0001 and p=0.0011, respectively) nearly a complete growing season after fertilization. These two effects accounted for most of the variation (r^2=0.87). Mean tree height differed as much as 0.31 m between fertilization treatment T4 (2.94 m) and T6 (2.63 m) (Table 4.2). Mean tree height was similar among fertilization treatments receiving applications of NH_4 (T2, T4, and T5 with the exception of T3). Treatments T1 and T6 (control and NO_3 plus P treatment) were significantly shorter.

Table 4.2. Tree heights of slash pines among six fertilization treatments before and after fertilization.

Block	Fertilization treatment N	P	Tree height Prefert.[a]	Postfert.[b]
	Kg/ha		Meters	
T1	0	0	1.68	2.73 c
T2	168.12 NH_4	0	1.70	2.93 a
T3	448.32 NH_4	0	1.58	2.85 b
T4	168.12 NH_4	112.08	1.56	2.94 a
T5	448.32 NH_4	112.08	1.67	2.92 a
T6	448.32 NO_3	112.08	1.71	2.63 d

a/Differences among fertilization treatments statistically non-significant.

[b]Least-squares means comparison presented below. Means with same letter are not significantly different.

Least-squares comparisons of adjusted postfertilization tree height means.

I/J[a]	Fertilization treatment T1	T2	T3	T4	T5	T6
T1	.	0.0001	0.0001	0.0001	0.0001	0.0001
T2		.	0.0001	0.6615	0.6193	0.0001
T3			.	0.0001	0.0004	0.0001
T4				.	0.3547	0.0001
T5					.	0.0001

[a]Prob. > |T| Ho: LSmean(I) = LSmean(J). At an assumed experimentwise error rate of E = 0.05, only differences with associated comparison probabilities less than 0.01 should be considered statistically different.

Table 4.3. Foliar nitrogen concentrations of slash pines among six fertilization treatments before and after fertilization.

Fertilization treatment			Foliar N concentration[a/]	
Block	N	P	Prefert.	Postfert.[b/]
	Kg/ha		Percent	
T1	0	0	0.809 b	1.020 d
T2	168.12 NH_4	0	0.872 a	1.307 c
T3	448.32 NH_4	0	0.878 a	1.605 b
T4	168.12 NH_4	112.08	0.805 b	1.481 b
T5	448.32 NH_4	112.08	0.783 b	1.983 a
T6	448.32 NO_3	112.08	0.764 b	1.468 b

[a/]Means in columns with the same letter are not significantly different (p=0.05).

[b/]Least-squares means comparison presented below.

Least-squares comparisons of adjusted postfertilization means of foliar N concentration

	Fertilization treatment					
I/J[a/]	T1	T2	T3	T4	T5	T6
T1	.	0.0001	0.0001	0.0001	0.0001	0.0001
T2		.	0.0001	0.0010	0.0001	0.0018
T3			.	0.1044	0.0001	0.1412
T4				.	0.0001	0.9640
T5					.	0.0001

[a/]Prob. > |T| Ho: LSmean(I) = LSmean(J). At an assumed experimentwise error rate of E = 0.05, only differences with associated comparison probabilities less than 0.01 should be considered statistically different.

Table 4.4. Foliar phosphorus concentrations of slash pines among six fertilization treatments before and after fertilization.

Fertilization treatment			Foliar P concentration[a]	
Block	N	P	Prefert.	Postfert.[b]
	Kg/ha		Percent	
T1	0	0	0.0609 c	0.0915 c
T2	168.12 NH_4	0	0.0706 a	0.0711 cd
T3	448.32 NH_4	0	0.0688 ab	0.0673 d
T4	168.12 NH_4	112.08	0.0661 b	0.1458 b
T5	448.32 NH_4	112.08	0.0668 ab	0.1654 a
T6	448.32 NO_3	112.08	0.0598 c	0.1561 ab

[a]Means in columns with the same letter are not significantly different (p=0.05).

[b]Least-squares mean comparison presented below.

Least-squares comparisons of adjusted postfertilization means of foliar P concentration.

	Fertilization treatment					
I/J[a]	T1	T2	T3	T4	T5	T6
T1	.	0.0339	0.0037	0.0001	0.0001	0.0001
T2		.	0.4088	0.0001	0.0001	0.0001
T3			.	0.0001	0.0001	0.0001
T4				.	0.0011	0.3010
T5					.	0.0356

[a]Prob. > |T| Ho: LSmean(I) = LSmean(J). At an assumed experimentwise error rate of E = 0.05, only differences with associated comparison probabilities less than 0.01 should be considered statistically different.

Foliar N and P response to fertilization. Foliar N and P concentrations showed a strong and significant response to fertilization treatment (p=0.0001 and p=0.0001, respectively) adjusting for initial variation. Foliar N and P concentrations nearly doubled in many fertilization treatments with differences corresponding to treatment application rates (Tables 4.3 and 4.4). As with postfertilization tree height, a high coefficient of determination (r^2=0.87 and r^2=0.93 for foliar N and P concentrations, respectively) indicated the strong relationship in foliar N and P levels with fertilization treatment.

Fertilization influences on foliar N and P concentrations are also evident in relation to expected growth response when plotted on a CRIFF (1985) modified Cate-Nelson curve (Figure 4.3). Increasing applications of NH_4 (T2 and T3) alone shifted foliar N concentrations from questionable sufficiency for trees in the control fertilization treatment (T1) to sufficient N and deficient P levels. Foliar N concentrations for treatments T1, T2, and T3 were statistically different from each other (Table 4.3). In these fertilization treatments, as the concentration of foliar N increased, P concentrations decreased. Foliar N and P concentrations of trees fertilized with 168.12 kg/ha N-NH_4 (T4) and 448.32 kg/ha N-NO_3 (T6), both with 112.08 kg/ha P, were similar. Trees in fertilization treatment T5, receiving the highest levels of fertilizer elements, had significantly higher concentrations of foliar N and P than all other treatments.

Characterization of Stem Tissue Response Types to Inoculation

Controls. A total of 418 sterile water controls (obtained from 209 branches) were evaluated for tissue response symptoms and cultured for the Fusarium subglutinans fungus (Table 4.5). Immune-like response was expressed in 96.2% of

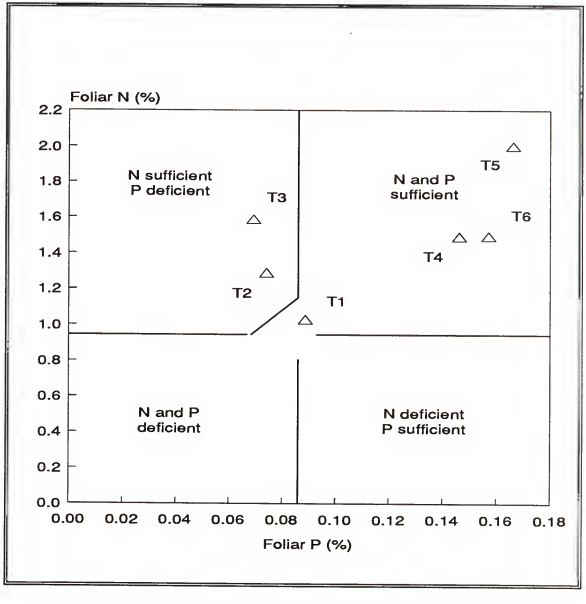

Figure 4.3. Postfertilization foliar N and P concentrations of slash pines according to expected tree growth (CRIFF 1985).

Table 4.5. Tissue response of slash pine branches to inoculations with sterile water.

Stem tissue response type	Control inoculations		Total
	Uninfected	Infected[a]	
		Frequency	
Immune-like	395	7	402
Callus	8	2	10
Diffuse canker	0	6	6
Total	403	15	418

[a]Fusarium subglutinans isolated and pine pathogenicity verified.

all controls. Fusarium subglutinans was isolated and pathogenically verified from 3.6% (15) of the control wounds. It is interesting to note that nine of the 15 infected control inoculations were made on the same day.

Within branch comparisons of inoculated tissue response. The same tissue response type occurred for both inoculations on nearly 83% (2405 of 2901) of the double inoculated branches (Table 4.6). The greatest difference (16.7%) in tissue responses on the same branch was between the callus and immune-like categories. This difference accounted for the significant variation (p=0.0001) between opposing inoculations. Opposing differences between diffuse canker and callus or immune-like categories were minimal (0.3% and 0.1% of the total branches, respectively).

Pathogen isolation and pathogenicity verification. Fusarium subglutinans was isolated from 86.1% of 2898 branches inoculated with the pitch canker fungus (Table 4.7). When separated according to tissue response type of the inoculated

Table 4.6. Tissue response within individual slash pine branches double-inoculated with the pitch canker fungus.

Difference in stem tissue response type	Frequency	Percent
None (same response)	2405	82.9
Immune-like vs. callus	485	16.7
Callus vs. diffuse canker	8	0.3
Immune-like vs. diffuse canker	3	0.1
Total	2901	100.0

branch, Fusarium subglutinans recoveries were highest from branches with diffuse cankers (95.7%), intermediate (87.7%) from immune-like responses, and lowest (74.9%) from callus response. Pine pathogenicity was verified for all (100%) cultures recovered from diffuse canker and immune-like responding branches (symptom expression extremes).

Influences of Host Genotype, Fungus Pathotype, and Site Fertilization

Site fertilization. The amount of branch dieback response to inoculation differed significantly among fertilization treatments (p=0.0001) ranging from 21.3% (T1) to 66.1% (T5) (Figure 4.4 and Table 4.8). The percentage of branch dieback significantly increased with increasing rates of N, but only when applied in combination with P (fertilization treatments T4, T5, and T6) (Table 4.9). Comparing

Table 4.7. Isolation and pine pathogenicity verification of Fusarium subglutinans from inoculated slash pine branches.

Stem tissue response	Pathogen isolation		Pine pathogenic verification	
	Freq.	% Pos.	Freq.	% Pos.
Immune-like	840	87.7	215	100.0
Callus[a]	1029	74.9	-	-
Diffuse canker[b]	1029	95.7	205	100.0
Total	2898		420	

[a]Pathogenicity not verified for callus response type.

[b]Isolation were not performed for three branches with diffuse canker stem tissue response type.

fertilization treatments receiving 168.12 kg/ha N-NH_4, amounts of branch dieback increased by more than 70% when 112.08 kg/ha P was applied (24.1% vs. 38.7% for T2 and T4, respectively). Branch dieback increased nearly three-fold between treatments receiving 448.32 kg/ha N-NH_4 without and with P (24.7% vs. 66.1% for T3 and T5, respectively). Comparing the effects of nitrogen form, the amount of branch dieback was significantly greater in those trees receiving applications of NH_4 than those receiving the same rate of N as NO_3 (66.1% vs. 43.3% for T5 and T6, respectively). Applications of 168.12 and 448.32 kg/ha N-NH_4 alone (T2 and T3, respectively) did not result in significant increases in branch dieback (24.1% and 24.7%, respectively) above ambient site fertility (T1) (21.3%) (Table 4.9).

The percentage of branches expressing the immune-like response appeared inversely related to branch dieback response (Figure 4.5). Fertilization treatment

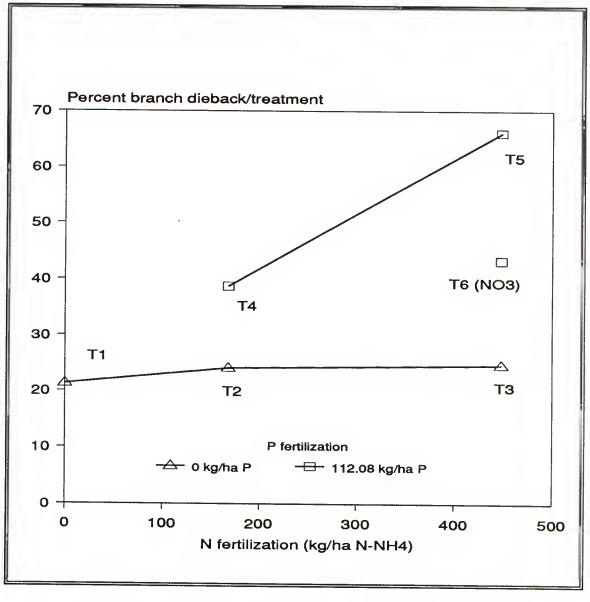

Figure 4.4. Branch dieback of slash pines inoculated with the pitch canker fungus among six fertilizer treatments.

Table 4.8. Effects of fertilization treatment, slash pine progeny, and pitch canker pathotypes on branch dieback.

Analysis of variance[a]				
Source	df	SS	F value	Pr. > F
Fertilization	5	29.95	151.07	0.0001
Rep.(Fert.)[b]	12	4.56		
Progeny	11	29.97	11.20	0.0001
Fert.*Progeny	55	11.43	0.85	0.7439
Prog.*Rep.(Frt.)[c]	132	32.12		
Pathotype	2	1.11	14.01	0.0001
Fert.*Pathotype	10	0.27	0.67	0.7518
Prog.*Pathotype	22	1.60	1.83	0.0143
Fert.*Prog.*Path.	110	3.85	0.88	0.7739
Sub-sub plot error	288	11.42		

[a]Proportion branch dieback analyzed as the angular transformation.

[b]Whole plot error.

[c]Sub-plot error.

T1, with the lowest amount of branch dieback (21.3%), had the greatest amount of immune-like response (47.7%). Conversely, T5 had the highest branch dieback (66.1%) and the lowest immune-like response (4.6%).

The amount of callus formation appeared relatively consistent among fertilization treatments and unrelated to levels of either branch dieback or

Table 4.9. Branch dieback of slash pines inoculated with the pitch canker fungus among six fertilization treatments.

Block	Fertilization treatment N	P	Branch dieback[a]
	Kg/ha		Percent
T1	0	0	21.3 a
T2	168.12 NH_4	0	24.1 a
T3	448.32 NH_4	0	24.7 a
T4	168.12 NH_4	112.08	38.7 b
T5	448.32 NH_4	112.08	66.1 c
T6	448.32 NO_3	112.08	43.3 b

[a]Means with the same letter are not significantly different (p=0.05).

immune response (Figure 4.5). Callus formation ranged from 29.7% for fertilization treatment T5 to 42.4% for treatment T3.

Host genotype. Slash pine progenies varied significantly (p=0.0001) in the percentage of branch dieback from 13.6% to 65.8% (Figure 4.6 and Table 4.8). Overall, host progenies G11 and G12 were significantly more susceptible and G1 less susceptible when evaluated as the percentage of branch dieback (65.8%, 59.0%, and 13.6%, respectively) (Table 4.10). Such distinction was not evident among the other host progenies, but represented a near continuum in frequencies of branch dieback (range 18.6% to 43.7%).

The percentage of branches expressing the immune-like response appeared inversely related to branch dieback among progeny (range 10.5% to 57.6% for progenies G12 and G1, respectively) (Figure 4.6). The percentage of callus formation appeared unrelated to amounts of branch dieback or immune-like response (range 25.0% to 49.0% for progenies G12 and G2, respectively).

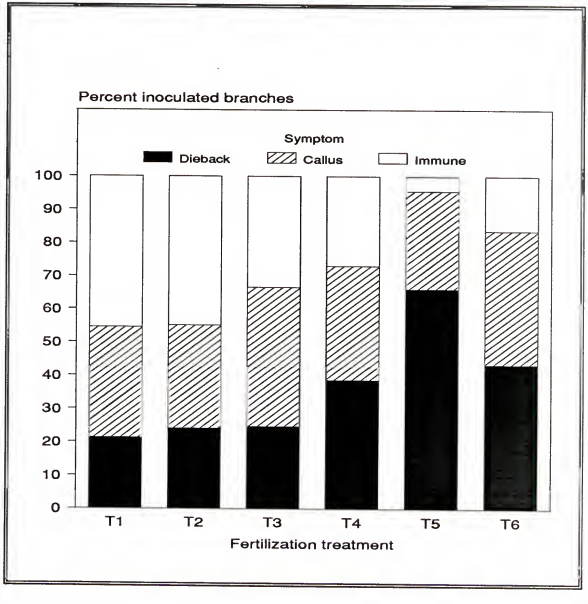

Figure 4.5. Branch dieback, callus formation, and immune-like response of slash pines inoculated with the pitch canker fungus among six fertilizer treatments.

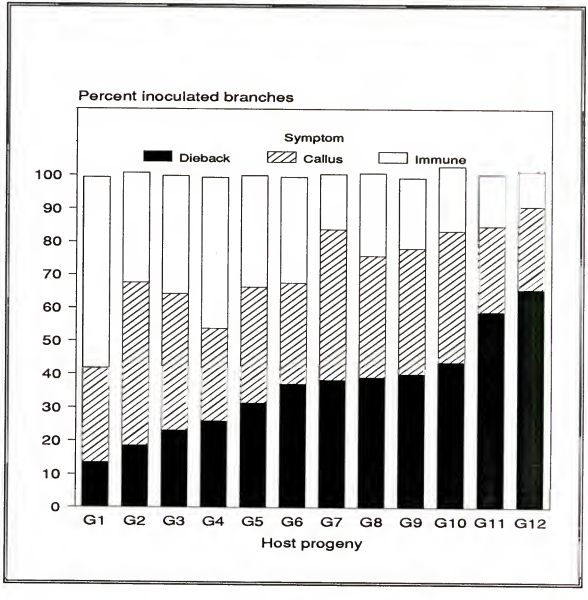

Figure 4.6. Branch dieback, callus formation, and immune-like response among 12 slash pine progenies inoculated with the pitch canker fungus.

Table 4.10. Branch dieback of 12 slash pine progenies inoculated with the pitch canker fungus.

Overall		Fungus pathotype[a]					
		P1		P2		P3	
Progeny	Mean[b]	Progeny	Mean	Progeny	Mean	Progeny	Mean
		Percent branch dieback					
G1	13.6 a	G1	11.7 a	G1	12.2 a	G1	16.9 a
G2	18.6 ab	G2	17.7 ab	G2	18.2 ab	G2	20.1 a
G3	23.2 bc	G3	22.8 bc	G3	25.0 bc	G3	21.9 a
G4	26.0 bcd	G4	25.7 bcd	G4	25.5 bc	G4	26.7 a
G5	31.5 cde	G5	32.5 bcd	G7	34.5 cd	G5	27.8 ab
G6	37.1 def	G6	32.3 bcd	G5	34.4 cd	G6	41.4 bc
G7	38.4 ef	G9	33.1 bcd	G8	36.3 cd	G7	45.0 c
G8	39.1 ef	G8	35.1 cd	G9	35.6 cd	G8	45.9 c
G9	40.1 ef	G7	35.7 cd	G6	37.5 cd	G9	51.7 c
G10	43.7 f	G10	38.0 d	G10	39.4 de	G10	53.7 cd
G11	59.0 g	G11	56.9 e	G11	52.0 ef	G11	68.1 de
G12	65.8 g	G12	62.7 e	G12	65.2 f	G12	69.4 e
Prob. > F			0.0001		0.0001		0.0001

[a]Fungus pathotype source: P1 = Gilchrist, 1982; P2 = Volusia, 1977; P3 = Volusia, 1982.

[b]Means in columns with the same letter are not significantly different (p=0.05).

Overall, host progenies responded similarly in the percentage of branch dieback among fertilization treatments (Table 4.8). Individually, however, fertilization treatment T5 (448.32 kg/ha N-NH_4 and 112.08 kg/ha P) appeared to interact with the more susceptible progenies (G3-G12) resulting in large increases in branch dieback compared to that of the other treatments (Figure 4.7). Branch dieback increased nearly three-fold from treatment T4 to T5 for progeny G3 (22.8% and 68.9%, respectively); however, branch dieback was nearly equal comparing the same treatments for the next most resistant progeny G2.

Host progeny significantly interacted with fungus pathotype (p=0.0143) (Table 4.8). When evaluated separately by pathotype, the effects of host progeny were also significant in each case (p=0.0001 for each pathotype) (Table 4.10). The percentage of branch dieback in the more susceptible host progenies (G6-G12) were higher when inoculated with pathotype P3 than with P1 and P2 (Figure 4.8 and Table 4.10). The most resistant progenies (G1-G4) were not noticeably responsive to pathotype. Strict ranking of the intermediate responding progenies (G5-G9) varied according to pathotype but these differences were not distinct for pathotypes P1 and P2 and only somewhat so for pathotype P3 (Table 4.10).

Fungus pathotype. There was a significant (p=0.0001), but small, overall difference in the amount of branch dieback caused by different pathotypes (ranging from 33.7% to 40.7% for pathotypes P1 and P3, respectively) (Figure 4.9 and Table 4.8). The amount of branch dieback associated with pathotype P3 was significantly higher than the other two pathotypes which were similar to each other (Table 4.11).

The significant host progeny x fungus pathotype interaction was the result of a significant increase in the percentage of branch dieback of intermediate and

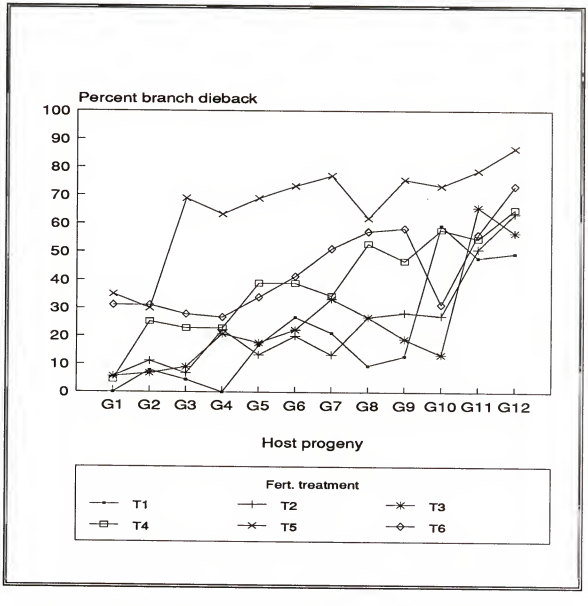

Figure 4.7. Branch dieback of 12 slash pine progenies inoculated with the pitch canker fungus among six fertilizer treatments.

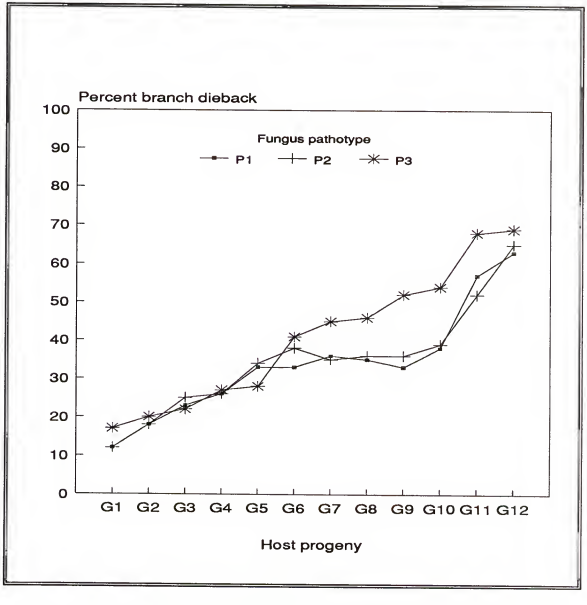

Figure 4.8. Branch dieback of 12 slash pine progenies among three pathotypes of the pitch canker fungus.

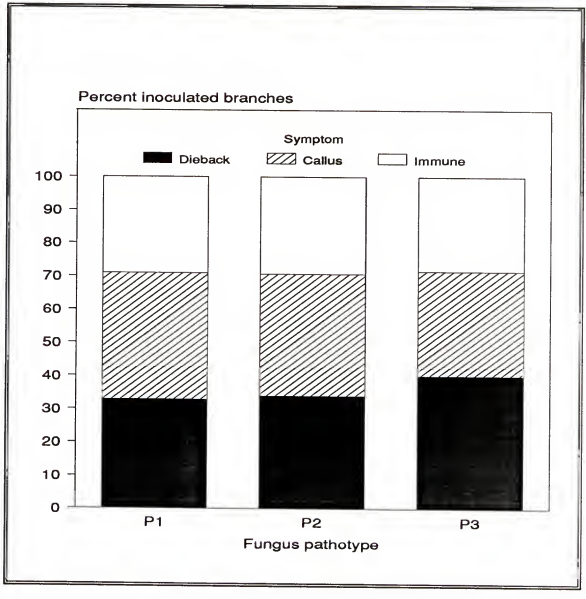

Figure 4.9. Branch dieback, callus formation, and immune-like response of inoculated slash pines among three pathotypes of the pitch canker fungus.

Table 4.11. Branch dieback of slash pines inoculated with three pathotypes of the pitch canker fungus.

Fungus pathotype	Branch dieback[a]
	Percent
P1 (Gilchrist, 1982)	33.7 a
P2 (Volusia, 1977)	34.6 a
P3 (Volusia, 1982)	40.7 b

[a]Means with the same letter are not significantly different (p=0.05).

susceptible progenies (G8-G11) to pathotype P3 compared to the other pathotypes (presented in Host genotype) (Figure 4.8 and Table 4.12).

Differences in the amounts of callus formation (less than 10%) appeared inversely related to levels of branch dieback and amounts of the immune-like response was nearly consistent among pathotypes (Figure 4.9).

Logistic Evaluation of Branch Dieback as a Function of Host Nutrition, Host Genotype, Fungus Pathotype, and Tree Height.

A significant logistic model was selected evaluating the relationships of fertilization treatment, foliar element content, host progeny, fungus pathotype, and tree height with branch dieback response. Among the 10 foliar elements evaluated, including all possible first and second order interactions, foliar concentrations of N and P (providing a direct measure of fertilization treatment effectiveness on the sample unit and therefore not influenced by spatial effects) were identified as significant components (p=0.0001 for both N and P) in the logistic

Table 4.12. Branch dieback of 12 slash pine progenies inoculated with three pathotypes of the pitch canker fungus.

Fungus pathotype[a]	Host progeny					
	G1	G2	G3	G4	G5	G6
	Percent branch dieback					
P1	11.7	17.7	22.8	25.7	32.5	32.3
P2	12.2	18.2	25.0	25.4	37.5	35.6
P3	16.9	20.1	21.9	26.7	27.8	41.1
Prob. > F	0.1196	0.6498	0.4956	0.9988	0.0646	0.1358

Fungus pathotype[a]	Host progeny					
	G7	G8	G9	G10	G11	G12
	Percent branch dieback[b]					
P1	35.7	35.1 a	33.1 a	38.0 a	56.9 a	62.7
P2	34.4	34.5 a	36.3 a	39.4 a	52.0 a	65.2
P3	45.0	45.9 b	51.7 b	53.7 b	68.1 b	69.4
Prob. > F	0.4692	0.0348	0.0001	0.0035	0.0186	0.2531

[a]Fungus pathotype source: P1 = Gilchrist, 1982; P2 = Volusia, 1977; P3 = Volusia, 1982.

[b]Means in columns with the same letter are not significantly different (p=0.05).

Table 4.13. Logistic regression model of host nutrition, host susceptibility groups, and pathotype virulence groups on branch dieback of slash pine inoculated with the pitch canker fungus.

Probability of branch dieback = $e^x / (1 + e^x)$

where: $x = -5.978 + 2.975(S1) + 1.867(S2) + 1.011(S3) + 0.0(S4) + 0.0(V1) + 0.358(V2) + 1.385(\%N) + 12.047(\%P)$

Variable	Beta[a]	Chi-square	P
Intercept	-5.978	329.22	<0.0001
S1[b]	2.975	167.2	<0.0001
S2	1.867	78.04	<0.0001
S3	1.011	20.75	<0.0001
S4	0.0	.	.
V1[c]	0.0	.	.
V2	0.358	15.37	<0.0001
N percent[d]	1.385	71.90	<0.0001
P percent[d]	12.047	104.69	<0.0001
Model		618.38	<0.01

[a]Regression coefficient.

[b]Host genotype susceptibility group: S1 = G11-G12; S2 = G5-G10; S3 = G2-G4; S4 = G1.

[c]Fungus pathotype virulence group: V1 = P1-P2; V2 = P3.

[d]Foliar element concentration.

Table 4.14. Least significant differences in branch dieback among 12 slash pine progenies inoculated with the pitch canker fungus.

Significant difference probabilities[a]

	Host progeny[b]											
I/J	G1 a	G2 b	G3 b	G4 b	G5 c	G6 cd	G7 cd	G8 cd	G9 cd	G10 d	G11 e	G12 e
G1	.	0.0010	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
G2		.	0.1644	0.0299	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
G3			.	0.4324	0.0006	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
G4				.	0.0082	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
G5					.	0.0799	0.0246	0.0149	0.0095	0.0001	0.0001	0.0001
G6						.	0.6179	0.4910	0.3965	0.0050	0.0001	0.0001
G7							.	0.8493	0.7268	0.0207	0.0001	0.0001
G8								.	0.8734	0.0336	0.0001	0.0001
G9									.	0.0492	0.0001	0.0001
G10										.	0.0001	0.0001
G11											.	0.0086

[a]Prob. > |T| Ho: LSmean(I) = LSmean(J). At an assumed experimentwise error rate of E = 0.05, only differences with associated comparison probabilities less than 0.005 should be considered statistically different.

[b]Progenies with same letter are not significantly different.

model and replaced the effects of fertilization treatment (Table 4.13). In this model, increases in the proportion of branch dieback were related to increases in concentration of both foliar elements independently (additive). Branch dieback was very sensitive to foliar P concentration levels.

Four groups of host genotypes were identified according to relative susceptibility based on the proportion of branch dieback response from a general linear

model of the significant effects previously evaluated in the logistic regression analysis (Table 4.14). These groups were classified as 1) susceptible (S1) - progenies G11 and G12; 2) intermediate (S2) - progenies G5-G10; 3) resistant (S3) - progenies G2-G4; and 4) very resistant (S4) - progeny G1. Each host susceptibility group was significant when evaluated in the logistic model (p=0.0001 for all groups) (Table 4.13). Accordingly, the probability of branch dieback increased as genotype group susceptibility increased in the logistic model.

Fungus pathotype P3 was also significant in relation to branch dieback response compared to pathotypes P1 and P2, however, this influence was not as strong as the other variables in the model (Table 4.13). Tree height growth was not statistically significant when added to the model.

Discussion

Host Response to Inoculation

One possible mechanism of pitch canker resistance expressed by slash pine appears to involve restricting pathogen invasion of host tissue. Symptom expression may be related to the rate the host responds to the presence of the pathogen. Callus produced adjacent to invaded host tissue may be similar to a wound response, perhaps under different genetic control. During containment by the host, the fungus may represent a chronic, low grade irritation eliciting callus formation. Rapid containment would result in minimal visible host response similar to the response elicited by wounding alone. If host response was slow or nonexistent, the fungus could colonize the entire branch before callus could be produced. Formation of visible callus may represent intermediate rates of host

response where a portion of host tissue is colonized before the pathogen is effectively contained, or it may possibly represent an artifact of the inoculation technique.

In this study, the immune-like response following inoculation with the pitch canker fungus appeared to represent an extreme case of this restrictive resistance mechanism. The pitch canker fungus was consistently recovered from these responses indicating containment of the pathogen by the host rather than a case of disease escape. The immune-like response was comparable to the wound response from inoculations with sterile water.

The external appearance of callus formation was apparently a less restrictive expression of the same resistance mechanism common to the immune-like response. Diffuse cankers rarely occurred opposite either callus or immune-like responses. Opposing differences between the immune-like response and callus formation, accounting for most of the differences of within branch inoculation types, also suggests that these responses represent different magnitudes of expression for the same resistance mechanism. Wounding from artificial inoculation may affect host tissues involved in pitch canker resistance. Differences in wounding, or host genetics controlling callus formation, may confound host response accounting for lack of relatedness of callus formation among treatments. The near absence of callus formation among the 418 control (water inoculated) branches, however, suggests that the observed callus response, with its distinctive darkened surface, is more closely associated with a host resistance mechanism than it is to a general response to wounding or a variation in wounding technique.

The amount of branch dieback was a significant indicator of differences in host susceptibility, pathogen virulence, and of specific host-pathogen combinations suggesting this expression is under strong genetic control. Because the

development of branch dieback is the cause of significant damage during pitch canker outbreaks, and this trait appears genetically conditioned, screening and breeding programs for pitch canker resistance using this criteria may be closely associated with field resistance.

Tree Height and Foliar Element Composition

Both precipitation and temperature during the study period did not substantially deviate from the 30 yr average. Therefore, results from this study did not appear to be influenced by extremes in these climatic factors.

Site influences were minor indicated by the strong and significant response in foliar N and P concentrations to fertilization treatment. Although there were significant prefertilization location differences in foliar N and P concentrations, the low coefficient of determination for these factors and the uniformity in prefertilization tree height indicated measured site influences were minimal. Furthermore, the prefertilization foliar N and P concentrations of trees in all fertilization treatment locations were considered limiting for slash pine growth (CRIFF 1985).

Site Fertilization

The amount of branch dieback of slash pine inoculated with the pitch canker fungus was strongly influenced by fertilization. The percentage of branch dieback significantly increased with increasing application rates of $N\text{-}NH_4$, but only when applied in combination with amendments of P, suggesting that influences of N application become important once sufficient P is available. Fertilization even

with 448.32 kg/ha $N\text{-}NH_4$ alone did not significantly affect branch dieback above control levels.

The amount of branch dieback was more closely related to levels of assimilated N and P in the host than fertilization treatment effects. Among the 10 foliar elements evaluated (and their interactions), only concentrations of foliar N and P were significantly related to branch dieback. Evaluation of the foliar elements in this manner accounted for spatial and genotypic effects in nutrient uptake within fertilization treatments.

The observed interactions of applied N and P on the amount of branch dieback appears to be the result of interactions between N and P assimilation. Branch dieback was very sensitive to foliar concentrations of P, and independent of foliar N concentrations. As applications of N alone increased, foliar N concentrations increased substantially while foliar P concentrations decreased slightly. Because of the sensitivity of branch dieback to foliar P concentrations, increases in the amount of branch dieback relating to increases in foliar N concentrations were counteracted by the decreases in foliar P concentrations. Therefore, influences of N fertilization alone on the amount of branch dieback appeared inconsequential when available P was limiting. Conversely, foliar P concentrations increased with increasing levels of N application once P was applied, thereby further increasing levels of branch dieback. It appears once sufficient P is available, increases in N availability promotes assimilation of P causing significant increases in the amount of branch dieback beyond the influences of increases in foliar N concentrations alone. Therefore, changes in site fertility that influence foliar N and P concentrations also appear capable of altering pitch canker susceptibility (as branch dieback). Specifically, applications of P alone may substantially increase pitch

canker susceptibility of slash pines growing on sites marginal in P availability but with adequate available N, and vice versa.

Levels of branch dieback for the 448.32 kg/ha $N\text{-}NO_3$ plus P treatment equalled that of the 168.12 kg/ha $N\text{-}NH_4$ plus P treatment. The similar foliar N concentrations between these plots indicates less N assimilated with fertilizer applications of $N\text{-}NO_3$ than $N\text{-}NH_4$. These results suggest differences in pitch canker disease expression are related to the quantity of N assimilated and not by nitrogen form as discussed by Huber and Watson (1974) for other plant diseases.

Influences of fertility and host nutrition on specific mechanisms governing disease development are poorly understood for most pathosystems. In slash pine, containment of pathogen invasion is one possible mechanism of pitch canker resistance. Thus, the amount of host tissue colonized would be determined by to the rate of pathogen invasion in relation to the rate of host response. Changes in host nutrition that can influence these rates would alter expressed susceptibility.

Altered host nutrition could influence pathogen aggressiveness by enhancing nutritional compatibility with the fungus through modification of the trophic environment. Specific changes could include pathogen growth, penetration, and enzymatic activity. Free amino acids and other derivatives such as monosubstituted guanidine compounds accumulate in conifer tissues in response to N fertilization (Durzan and Steward 1983). Certain monosubstituted guanidine compounds are excellent N sources for saprophytic fungi on fallen timber. Additional studies could determine the influence of nitrogenous sources in slash pine, that respond to N and P fertilization, on growth and development of the pitch canker fungus.

Changes in slash pine resistance to the pitch canker fungus influenced by nutrition could include reduction in the accumulation of inhibitory compounds

around infection sites. The effectiveness of host response would be reduced allowing greater pathogen colonization of host tissues before containment would occur (if at all). In host tissues, the pitch canker fungus elicits and grows in resin; known fungistatic to many fungi. Inhibitory compounds in slash pines relevant to pitch canker are unknown. Determining of the composition and rate of response of these compounds inhibitory to the pitch canker fungus, if they exist, could provide a basis for rapid evaluation of host susceptibility.

Fertilization with N and P may overcome the most prevalent nutrient deficiencies limiting growth, but may create a second order demand for other restricted nutrients that directly influence pitch canker expression (Huber 1980a, 1980b). If so, increases in pitch canker susceptibility associated with forest fertilization with N and P may be counteracted with additional amendments. Fisher et al. (1981) reported that increased mortality of previously infected slash pine from applications with high rates of N and P was significantly reduced following application of other essential elements including micronutrients. Therefore, the results from this study may differ according to nutrient availability of the site. Differences in nutrient availability among sites may also explain the inconsistencies in results among other studies evaluating the influence of fertilization on pitch canker (Anderson and Blakeslee 1984, Fisher et al. 1981, Fraedrich 1979, Fraedrich and Witcher 1982, Lowerts et al. 1985, Phelps and Chellman 1976, Wilkinson et al. 1977). Additional studies are needed to evaluate the influences of other nutrients in developing comprehensive site fertility strategies integrating pitch canker management.

It is not possible to determine from this study whether the expression of pitch canker susceptibility is directly altered by foliar N and P concentrations or if this apparent relationship actually consists of additional independent factors

responding in a similar manner to changes in site fertility. Whether independent or directly related, this relationship may provide the basis for further studies to develop a means to evaluate potential changes in pitch canker susceptibility important in slash pine selection and breeding and for seed orchard and plantation management decisions involving fertilization.

Host Genotype

Levels of branch dieback varied widely among all slash pine progenies relating to differences in pitch canker susceptibility. Quantitatively, these differences suggest potential for genetic gain in pitch canker resistance through selection and breeding.

Although overall host progeny x fertilization treatment interactions were not significant, slash pine progenies intermediate in branch dieback showed considerable sensitivity to fertilization influences. Except for the two most resistant progenies (G1 and G2), fertilization with 448.32 kg/ha N-NH4 and 112.08 kg/ha P (T5) increased branch dieback among all progenies to levels nearly equal to the most susceptible progenies (G11 and G12). These results indicate pitch canker susceptibility of the more resistant slash pine progenies is relatively stable over a range in nutrient availability and such progenies may be better integrated with forest fertilization without substantially increasing disease hazard. Fusiform rust susceptibility of loblolly and slash pine was unaltered by fertilization in resistant families while disease in susceptible families was significantly increased (Hollis et al. 1977, Powers and Rowan 1983).

Pitch canker disease expression (as the percentage of branch dieback) in slash pine represents the combined expression of nutritional and genetic factors,

as well as the effects of other response-regulating factors not identified in this study. Results from the logistic model suggest the continuous range in phenotypic expression of branch dieback among half-sib progenies consist of four genotype susceptibility groups highly sensitive to factors influencing foliar concentrations of N and especially P. Studies identifying additional response-regulating factors are required to further define the genetic components in the phenotypic expression of pitch canker resistance in slash pine.

Results from this study demonstrate that selection and breeding programs for pitch canker resistance should consider site fertility differences. Pitch canker resistance appears unrelated to tree height, indicating favorable selection and breeding potential in slash pine for components of growth response to fertilization and pitch canker resistance.

Fungus Pathotype

Statistically, differences in the amounts of branch dieback among pitch canker pathotypes were significant when evaluated with both the analysis of variance and the logistic model; however, these differences may not reflect important genetic differences in virulence. Differences in the amounts of branch dieback, callus formation, and immune-like responses were less than 10% among pathotypes, and the coefficient for pathotype effects in the logistic model was relatively minor.

On an individual progeny basis, differences in pathogen virulence were significant, but only for the more susceptible progenies. These differences demonstrate the importance of inoculum selection when evaluating pitch canker expression using artificial inoculation procedures. Furthermore, if there are geographic

differences in pathotypes, then artificial inoculation may provide a more efficient method to expose host genotypes than geographically dispersed field tests. As such, however, these observed differences do not seem large enough to fully account for source differences in pitch canker disease expression, but they do suggest that biologically significant variation may exist among a larger population of the pitch canker fungus.

Summary

In field experiments, fertilization of slash pine with up to 448.32 kg/ha N-NH_4 alone did not significantly increase amounts of branch dieback on sites deficient in P and N for growth. Levels of branch dieback significantly increased with increasing NH_4 application rates once sufficient P became available. The amount of branch dieback was strongly associated with foliar P concentrations. Foliar N concentrations were also significantly associated with branch dieback but independent of foliar P levels. Interactions in assimilation between N and P in response to different fertilization treatments appear to explain the observed interactions of applied N and P on host susceptibility. Foliar P concentrations decrease with increasing applications of N alone, but once sufficient P was available, foliar concentrations of P increased with increasing N availability. Differences in amounts of branch dieback between comparable rates (448.32 kg/ha N and 112.08 kg/ha P) of different N forms (NH_4 vs. NO_3) also appeared related to differences in the quantity of foliar N. Incidence of branch dieback of half-sib slash pine progenies intermediate in susceptibility appeared most sensitive to changes in site fertility while those most resistant and susceptible were relatively unaffected.

Phenotypic expression of branch dieback for slash pines artificially inoculated with the pitch canker fungus appears under genetic control, but expression is modified by environmental conditions capable of influencing foliar concentrations of N and P. Four host susceptibility groups were identified once foliar N and P concentrations and pathotype effects were accounted for. One possible mechanism for resistance appears related to the rate of host containment of the pathogen in infected tissues.

Differences in virulence among pathotypes of the pitch canker fungus were significant only on the more susceptible progenies demonstrating the importance of pathotype in evaluating pitch canker susceptibility for selection and breeding. The minimal overall differences among pathotypes did not indicate source differences in pitch canker expression but suggest biologically significant variation may exist in a larger pathogen population.

Results from this study demonstrate the potential for effective management of pitch canker in slash pine plantations through the manipulation of environmental factors capable of influencing foliar N and P concentrations and genetic factors governing pitch canker resistance and nutrient assimilation.

CHAPTER V

SUMMARY AND CONCLUSIONS

Genetic and environmental factors regulating the expression of pitch canker disease in slash pine were examined. Host responses to pitch canker inoculum sources and concentrations, wounding techniques, and seasons of inoculation were evaluated among four mature slash pine seed orchard clones.

Placing aqueous conidial suspensions of Fusarium subglutinans into branch wounds made by piercing the outer bark of slash pine branches with a hypodermic needle provided consistent exposure of host tissue to the pitch canker pathogen. Results from this inoculation technique were used to estimate relative levels and types of host responses. Successful inoculation of wounds (expressed as percentage of branch dieback) made by removing needle fascicles was less reliable. Branch dieback rarely occurred when inoculum was placed on the branch in the absence of wounding confirming Fusarium subglutinans as a wound-infecting pathogen.

Phenotypic expression of pitch canker-infected slash pine appears under strong genetic control. Amounts of branch dieback among seed orchard clones, artificially inoculated with the pitch canker fungus, varied significantly from nearly immune (0.0%) to very susceptible (57.3%). Ramets within each clone were relatively consistent in response.

The basis for pitch canker resistance appears to be regulated by internal mechanisms including those related to the ability of the host to contain the pathogen within relatively small areas of infected tissue. In the absence of branch

dieback, a darkened area of hypertrophic callus tissue was commonly observed surrounding the inoculation site. Production of callus in response to inoculation may be similar to the wound response and perhaps under different genetic control than the host response restricting pathogen invasion. Invasion of host tissues by the pathogen may represent an irritation capable of eliciting a wound response. Callus formation was minimal on the resistant and susceptible seed orchard clones suggesting that in the former case, host response rate was rapid thereby arresting pathogen invasion and in the latter case, the host response was slow allowing for successful pathogen invasion. Pine pathogenic Fusarium subglutinans was consistently recovered from all host response types indicating disease escape did not account for the observed response differences.

Pitch canker susceptibility estimates of slash pine clones from two different survey techniques of naturally occurring pitch canker crown infections agreed with artificial inoculation results. These results suggest collection of seed by clone, or roguing of seed orchard clones based upon these survey techniques, may provide substantial genetic gains in pitch canker resistance providing there is reasonable levels of heritability and if the seed orchard environment presents a pitch canker hazard equal to or greater than plantation conditions.

Disease expression of susceptible slash pine tissue appeared directly related to the concentration of inoculum. Levels of branch dieback increased with increasing inoculum concentrations although these effects were statistically significant only for the most susceptible clone. Although appreciable levels of branch dieback resulted from inoculations with concentrations of 1×10^2 conidia/ml, inoculum concentrations of 1×10^4 conidia/ml and greater produced consistent symptom expression required for reliable estimations of pitch canker susceptibility.

Differences in the percentage of branch dieback resulting from inoculations with different isolate sources were statistically significant only for a specific pathogen source-host clone combination indicating the importance in selecting inoculum source for evaluating pitch canker susceptibility of slash pine. Relationships were not apparent, however, between the amounts of branch dieback from inoculation and levels of disease expression in the stand from which the inoculum source was obtained suggesting disease outbreaks may be regulated more by environmental factors than by differences in pathogen virulence.

Amounts of branch dieback did not significantly differ between summer and fall inoculations. Seasonal differences in susceptibility of the host may be significant, however, during other seasons or with greater time intervals between inoculations.

Influences of nitrogen and phosphorus fertilization rates and nitrogen forms on symptom expression of open-pollinated slash pine progenies inoculated with different pitch canker pathotypes were evaluated in a separate field study. Amounts of branch dieback increased significantly with increasing amounts of ammonium sulfate (168.12 and 448.32 kg/ha N) only when applied in combination with concentrated superphosphate (112.08 kg/ha P) on sites deficient or marginal in both elements for growth of slash pine. Applications of ammonium sulfate alone, regardless of rate, did not significantly affect the amount of branch dieback.

Amounts of branch dieback were more closely related to concentrations of foliar N and P than fertilization treatment indicating N and P assimilation and changes in pitch canker susceptibility are closely associated. The amount of branch dieback was very sensitive to changes in foliar P concentrations and independent to N effects once concentrations of both elements exceeded levels limiting to growth (0.95% and 0.085% for N and P, respectively). Therefore,

applications of N alone on sites with sufficient P availability, or vice versa, may substantially increase pitch canker susceptibility. Foliar N and P concentrations and levels of branch dieback were similar between fertilization treatments receiving 168.12 kg/ha $N\text{-}NH_4$ and 448.32 kg/ha $N\text{-}NO_3$, both with concentrated superphosphate (112.08 kg/ha P), indicating effects of N form on branch dieback were related to the quantity of N assimilated.

The amount of branch dieback varied significantly among the half-sib slash pine progenies. From the apparent continuum in levels of branch dieback expressed among the progenies, four pitch canker susceptibility groups were identified when foliar P and N concentrations and pathotype effects were considered. These results indicate a strong genetic component in the phenotypic expression of pitch canker in slash pine.

Host responses of slash pine progenies intermediate in pitch canker susceptibility appeared most influenced by fertilization, although these differences were not statistically significant. Fertilization did not influence host response in more resistant or susceptible progenies. Further research evaluating additional progenies and fertilization treatments is required to identify such potential interactions.

The amount of branch dieback also significantly differed among pitch canker pathotypes, but these differences were less than 10%. Pathotypes significantly interacted with host progenies. Inoculations with one of the pathotypes consistently resulted in significantly greater levels of branch dieback, but only with the more responsive progenies. These results are in close agreement with the host clone x pathogen source interaction observed in the previous seed orchard experiment where pathogen source influences were significant only for the clone intermediate in susceptibility. The limited differences in host response among the

pathotypes evaluated in either of these studies, however, does not fully account for differences in pitch canker expression among the stands of origin, but does suggests biologically significant variation may exist among larger populations of the pitch canker fungus. Therefore, major disease outbreaks in slash pine plantations and seed orchards appear as the consequence of the genetic components of the host and predisposing environmental conditions such as site fertility.

Results from this study identify forest management considerations important in the fertilization of slash pine in areas where pitch canker is a potential concern. Slash pine progenies with high levels of pitch canker resistance appear unaffected by site fertility and could be used in conjunction with forest fertilization without increasing the susceptibility of the stand. In established slash pine seed orchards and plantations, decisions regarding fertilization and associated changes in host susceptibility could be evaluated on the basis of foliar N and P concentrations, just as growth response is now evaluated.

Further research is needed to evaluate those site and management factors, including additional forest fertilization practices, that significantly affect pitch canker disease development in order to develop comprehensive recommendations for intensive slash pine culture. Specifically, evaluation into the therapeutic potential of other nutrient amendments to reverse N and P fertilization associated increases in susceptibility may provide a means to ameliorate existing disease conditions or reduce disease hazard.

The ability to evaluate and predict disease hazard for a specific site or region would allow disease management decisions to help prevent pitch canker outbreaks. To achieve this objective additional research is required to identify edaphic components influential in mitigating disease potential (especially regarding host susceptibility).

Based upon observations of symptomatology under the conditions of this research, a major mechanism of host resistance to pitch canker may be the localized containment of the pathogen. Additional research into actual resistance mechanisms may provide methods to expedite progeny evaluations (e.g., tissue culture) and genetically enhance and manipulate pitch canker susceptibility.

APPENDIX
UNIVERSITY OF FLORIDA GENETICS COOPERATIVE IDENTIFICATION OF SLASH PINE GENOTYPES

	Host genotype reference	Genetics Coop. identification
Chapter III		
	116	120-56
	122	84-57
	205	222-57
	210	225-57
Chapter IV		
	1	55-59
	2	306-56
	3	58-59
	4	172-58
	5	28-57
	6	122-61
	7	46-62
	8	130-60
	9	49-57
	10	4-61
	11	32-59
	12	52-56

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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